VOLUME LXI NUMBER 4

THE
BOTANICAL GAZETTE

APRIL 1916

BIOCHEMICAL AND PHYSIOLOGICAL STUDY OF THE REST PERIOD IN THE TUBERS OF SOLANUM TUBEROSUM[1]

CHARLES O. APPLEMAN

(WITH TWO FIGURES)

Introduction

The cause and control of the rest period in plants have long been the subject of study, not only on account of their scientific interest, but also on account of the practical values which would accrue in many cases if the rest period were subject to control. Although many seeds have a rest period, this paper is concerned mainly with the rest periods in stems.

In spite of the immense amount of work that has been done on this problem, there still exist today two schools of thought, which stand directly opposed to each other on the first and most fundamental question involved, one school claiming that the rest period is a direct response to changing external conditions, while the other considers it to be the result of fixed, hereditary, internal causes. A middle ground is taken by others who attribute the rest period to a "cooperation of an hereditary tendency to rhythm, with the after effect of periodic repetition of reactions induced by external conditions." This diversity of opinion may be attributed largely to the variety of material used for the study of the problem and to the lack of exact and reliable experimental data.

[1] Contribution from the Laboratory of Plant Physiology, Maryland Agricultural Experiment Station. Published by permission of the Director.

Historical

As examples of the extreme views in the older literature, we may mention those of GRISEBACH and ASKENASY. The former considered the yearly periodicity of plants of the temperate regions entirely a hereditary property, induced probably by physiological selection due to alternating external conditions through a long series of years; whereas the latter asserted that it is due directly to external conditions. SACHS believed that the rest period is caused by a deficiency of soluble food, and the cessation of the rest is due to a gradual production of enzymes. He does not state whether internal or external causes are responsible for the deficiency of food, nor does he suggest the nature of the process which increases the enzymes. FISHER (**8**) studied the carbohydrate transformations in woody stems during the rest period, and concluded that periodicity of growth is conditioned by a periodicity of the processes of food changes, which in turn rest upon a hereditary periodicity of certain properties of the protoplasm.

Coming now to the more modern literature, the studies of MÜLLER-THURGAU and SCHNEIDER-ORELLI (**19–22**) are of importance in the solution of this problem. They think the winter rest of plants is not conditioned by low temperature only, but the cessation of growth rests also upon the internal properties of the protoplasm of the meristem. These internal properties are inherited effects of winter cold on the growth process which after a long time become fixed in the protoplasm.

HOWARD (**10**) experimented with a large number of trees and shrubs, all deciduous species native to the temperate zone. He first tested their ability to grow in winter under favorable greenhouse conditions and then subjected to artificial treatments those which failed. Discussing the results of the foregoing experiments, the author states: "This shows conclusively that the majority of species, indigenous to the temperate climates, do not have firmly fixed winter rest periods from which they cannot be awakened." Although his observations were made on plants in winter rest, he includes also summer rest periods in his general conclusions. He thinks both forms of rest are caused by unfavorable external conditions. If these conditions, such as cold or drought, occur at regu-

lar intervals, a plant readily adapts itself to the new demands and the rest becomes a habit, which may continue to be repeated automatically for a longer or shorter period of time. The habit of rest thus induced is often very strongly fixed and is apparently transmissible.

EULER (**7**) attributes the rest period to internal self-regulatory processes and theorized in the following ingenious manner regarding the nature of these processes. The growth of a young cell shows two phases which follow each other by self-regulation. The first or stretching phase shows an increase of soluble osmotically active cell constituents; the second is characterized by the building up of the soluble materials into insoluble or colloidal molecules. Hydrolysis and synthesis, however, proceed simultaneously, and the two phases are characterized by an excess of the one process over the other. The synthesis of the second phase fixes the stretching of the first phase. What applies to the single cell applies also to the development of the entire organ and organism. In a number of cases, new cycles of stretching and condensation follow one another with unbroken regularity. In many other cases, a new cycle does not immediately follow upon the ending of a previous one. The stretching phase is prevented on account of an inability to dissolve the highly complex reserve materials. A short or long rest period must first take place. Finally a point is reached, and this point determines the length of the rest period, when the synthetic processes no longer hold the simultaneously occurring hydrolytic processes in equilibrium, and as a result internal osmotic pressures are increased. The resting organ is now physiologically ripe and begins to germinate, external conditions being favorable. As long as condensation processes predominate in the resting organ, germination is impossible. According to EULER, therefore, after-ripening would simply consist in certain changes during the rest period which weaken the synthetic processes. This theory, however, does not explain the origin and character of these changes.

The important observations of SCHIMPER (**25**) gave us the first real knowledge of the peculiar behavior of tropical vegetation in respect to growth and rest periods. In places where moisture and temperature conditions are favorable during the entire year, many

plants show a rhythmic alternation of periods of rest and activity of the growth processes. SCHIMPER states: "Internal causes are mainly or solely responsible for the alternation of rest and activity in a nearly uniform climate. Such a rhythmic change, however, is never abandoned, for it arises from the nature of the living organism and not from external conditions; its connection with external conditions is a secondary feature, an adaptation."

From the time of SCHIMPER'S observations, tropical vegetation has been favorite material for study in determining the relation of external conditions to rest periods. KLEBS (**16**) has made observations on the tropical vegetation of Java, where climatic conditions show little variation. Exact measurements of twigs were made to determine the amount of growth over a series of months. He also cultivated a number of European and Japanese plants in Java. Native plants of Java were likewise transplanted to Heidelberg and grown in a greenhouse under constant temperature and moisture conditions. These studies, which constitute his most recent research on this problem, led him to deny firmly a periodicity of growth which is independent of external factors, and to formulate the following hypothesis to account for this rest period in tropical trees: When the growth ability is weakened by a deficiency of one or more of the essential external growth factors, carbon assimilation proceeds at first at the normal rate, resulting in a storage of organic materials. These in turn render certain enzymes inactive and thus cause a rest period. All means available for shortening the rest period simply activate these enzymes. Great stress is laid upon salts as the causal agent in the rest periods of tropical plants, since the other three factors are constantly favorable for growth in Java.

VOLKENS (**28, 29**) made more extended observations on the tropical vegetation in its natural habitat. Trees were marked and observed for an entire year. Many new and interesting facts were added to our stock of knowledge regarding rest and activity in tropical plants. Although his observations were made in the same place as those of KLEBS and often on the same object, he reached a very different conclusion. He is unable to see any relation between the rest periods in tropical plants and external conditions;

they must be due to internal causes. He does not intimate, however, the nature of these causes.

It is true that VOLKENS does not include the factor of salts among the external conditions that may affect the growth processes; and besides, he and KLEBS do not seem to have quite the same conception of internal causes, so that their views may not be as antagonistic as first seems. According to VOLKENS, everything is conditioned by internal causes which cannot be brought into evident connection with external factors. The important thing to KLEBS is whether the rest is caused by the outer world or is an expression of an internal fixed "specific structure." VOLKENS does not discriminate between specific structure and internal conditions. It is conceivable that a slight external change may set into motion a chain of effects which would ultimately so change the internal conditions of the growing cell or its immediate environment that growth would be arrested and seem to be due to internal specific structure.

Attempts to shorten the rest period of buds and bulbs by artificial means are numerous and some have been successful. MÜLLER-THURGAU (**20**) claims to have shortened the rest period of potato tubers by one month's storage at 0° C. The use of ether and chloroform dates from the important work of JOHANNSEN (**14**), who succeeded by their means in forcing buds to open 3–6 weeks earlier than normally. He found, however, that these agents are effective only at the beginning and near the end of the rest periods. The warm bath has been used successfully in shortening the rest period. MOLISCH (**18**) caused an earlier opening of buds by immersing the shoots in water at 35° C. MÜLLER-THORGAU and SCHNEIDER-ORELLI (**22**) hastened the growth of lily-of-the-valley bulbs and potato tubers by warming them at a temperature of 38° C. and maintaining a germination temperature of 26° C. Warm air was equally as effective as warm water. In an endeavor to test KLEBS's conception regarding the importance of salts in bud growth, LAKON (**17**) stood cut twigs of trees and shrubs in Knop's solution and found that of a large number only one failed to show an earlier unfolding of the buds than normally. This does not prove, however, that the buds failed to germinate on account of the lack of salts any more than the lack of ether prevented germination in

JOHANNSEN'S experiments. JESENKO (12) found that hydrochloric, sulphuric, and tartaric acids, as well as alcohol and water saturated with carbon dioxide, hastened the germination of buds of a number of woody plants. He concluded that the applied solutions not only acted as a stimulus in the strict sense of the word, but also started certain chemical processes in the buds which produced favorable conditions for growth. JOST (13) observed that wounded potatoes, especially if they were cut into many pieces, showed an earlier germination of the buds than unwounded ones.

WISMEWSKI (31) experimented with the winter buds of certain aquatic plants. The rest period of the buds of *Hydrocharis Morsus-ranae* could be shortened by wounding and lengthened by darkness. *Hydrocharis* formed buds the entire winter when kept in darkness and immersed in rainwater or a nutritive solution. He concludes that neither the origin nor germination of these buds is conditioned by an inner rhythm of the plants. He also states that low temperature is not a necessary condition for the origin of buds.

Although the rest period may be shortened by artificial treatments, none has thus far been capable of entirely eliminating the rest period where it is well fixed. A great deal of work has been done on forcing resting stem structures into growth by artificial means, but comparatively little has been done to determine the character of the physiological and chemical changes effected by such treatments. Still less is known about these changes during the natural rest period.

MÜLLER-THURGAU and SCHNEIDER-ORELLI (21, 22) found increased respiration in potato tubers and lily-of-the-valley bulbs after the warm-bath treatment and also after etherizing.

IRAKLIONOW (27) also found a rise in respiration of potato tubers after treatment with warm water. At the end of a few days, however, it fell back to the normal rate and did not rise again until the beginning of germination. He assumes, in agreement with MOLISCH, that the breaking of the rest period by means of the warm bath is a simultaneous action of the high temperature and the water, and that the warm bath influences the enzymes, chiefly the oxidases.

BUTKEWITSCH (4) showed that the stored starch in the cortex and wood can be dissolved by the action of toluol and chloroform, and also by high temperature. He thinks the action is similar to cold and consists in a weakening of the plastids.

GRÜSS (9) investigated the chemical changes set up in the cells around the wound in potatoes, and found an increase in the oxidizing enzymes and in the diastase activity. There was some sugar accumulation in the subphellogen. MÜLLER-THURGAU (19) was probably the first to observe the accumulation of sugar in potatoes stored at low temperatures.

FISHER (8) studied the changes in the starch content of trees during the rest period. He found the maximum of starch from leaf fall to the beginning of November. Starch solution began in November and reduced the starch content to the minimum in the winter. Starch synthesis began in March and reached the maximum again in April, after which hydrolysis began and brought the starch content to the minimum during the latter part of May, or the time of the beginning of vegetative activity. Storage of starch occurred of course during the summer.

Biochemical

It is obvious from the foregoing survey of the literature that there is little exact experimental data on the chemical and physical situation in stems which forces the growing cells into a period of rest. The same is true of the changes in the resting tissue or its immediate environment which are essential to the release of the growth processes at the end of the rest period. These latter changes will be spoken of as after-ripening, using the term in its broadest sense.

It is a well known fact that under normal conditions potato tubers will not sprout for several weeks after harvest. Rehobeth potatoes harvested on July 17 and planted immediately in the same field did not sprout until October, although good growing conditions prevailed during the entire period. McCormick potatoes harvested on November 11, and kept constantly under favorable growing conditions in the greenhouse, did not sprout until January 24; general sprouting did not occur until February. It has been gen-

erally conjectured that the after-ripening processes in tubers are metabolic in character; in other words, it has been supposed that chemical changes occur in the tubers during the rest period which render certain essential foods or other materials available for the growing bud. The first problem in the following study was to ascertain if there are metabolic processes occurring in the potato tuber during the rest period which are characteristic of after-ripening, and to determine their character as far as possible.

The tubers used in this investigation produce sprouts much earlier from the buds on the seed or terminal end when the tubers are left whole. In the majority of cases, the buds on the stem or basal end do not germinate at all unless those on the seed end are injured; therefore, tubers were cut in half at regular intervals during the rest period, and the following analyses made separately on the seed and stem halves with a view to detecting better the chemical changes characteristic of after-ripening. We are not justified in assuming that all chemical changes occuring in the whole tuber during the rest period must be peculiar to after-ripening.

CARBOHYDRATES.—In a previous chapter it was stated that storage at low temperature has been claimed to be capable of shortening the rest period of potato tubers. It has been proved that during such storage sugar accumulates in the tubers. These facts have led to the supposition that the rest period is caused by a deficiency of soluble carbohydrates, and that the cessation of the rest is due to a gradual production of the diastase. In order to determine if carbohydrate transformations are essential after-ripening processes, or simply due to changing temperature, analyses were made during after-ripening of tubers at a fairly constant and favorable growing temperature. At the same time analyses were made on tubers stored under variable low temperature in a potato vault. Samples of 8 tubers, each with a total weight of about one kilogram, were selected from both lots at intervals of 2 and 4 weeks. Each tuber was cut into two equal parts, the one part representing the terminal or seed end, the other, the basal or stem end. The seed ends of each sample were all grated together; likewise the stem ends. After being thoroughly mixed in a mortar,

both lots of pulp were sampled for determinations of moisture, starch, total sugar, reducing sugar, and diastase.[2]

The final carbohydrate results are calculated to original moisture basis. It is thus possible to record results in percentage of wet weight, as the possibility of apparent changes due to loss of water by evaporation is excluded. Apparent changes due to this water loss would also be excluded by calculating results to dry basis at the time of analysis, but these results might still show changes due to water taken up by hydrolysis, or to the accumulation of respiratory water, especially where evaporation is prevented. This method, however, does not take into account loss in dry matter through respiration. In the case of the vault-stored potatoes this would be negligible, since respiration is very low at the vault temperature.

Failure of growth in the buds during the rest period is not due to a lack of available sugar, since the percentage of both reducing and total sugars in the greenhouse-stored tubers was no greater when sprouting began than at the beginning of the rest period (tables I–IV); besides, the seed end at the time of sprouting did not contain a greater percentage of sugar than the stem end. *The carbohydrate transformations during the rest period are entirely dependent upon changing temperature, and must, therefore, not be considered after-ripening processes.*

DIASTASE.—Since the supply of soluble carbohydrates for the growing buds is dependent upon the important enzyme diastase, a gravimetric method was employed to determine the diastatic power of the juice from the two ends at intervals during after-ripening under greenhouse conditions. The determinations were made on the same samples used for the carbohydrate analyses.

The increase in total sugar after incubation was considered an index of the diastatic activity of the potato extract at the time of analysis. It may indicate simply the excess of the hydrolytic process over a simultaneously occurring synthetic process. On either basis, the potato extract contained active diastase at all times during the rest period. It was uniformly greater in the

[2] For detailed descriptions of methods, as well as additional tables and figures, see Bull. no. 183. Maryland Agric. Exper. Sta.

extract from the seed end, but there was no appreciable increase in the extract from either end during the rest period. It may be concluded, therefore, that the *cessation of the rest period is not due to a gradual increase of diastase activity.*

TABLE I

REDUCING SUGAR; MCCORMICK POTATOES PLANTED IN WET SAWDUST IN GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	REDUCING SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 14	.....	0.287	0.320	0.304
December 14	20° C. to 22° C.	0.372	0.342	0.357
January 20	22° C. to 17° C.	0.478	0.481	0.479
February 3	20° C. to 22° C.	0.274	0.311	0.293
March 26	21° C. to 23° C.	0.385	0.401	0.393

TABLE II

REDUCING SUGAR; GREEN MOUNTAIN POTATOES STORED IN VAULT

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	REDUCING SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 28	..... to 5° C.	0.655	0.745	0.70
December 20	5° C. to 2° C.	0.972	1.40	1.19
January 13	2° C. to −1.5° C.	1.35	1.85	1.60
February 13	−1.5° C. to − 1° C.	2.20	2.62	2.40

TABLE III

TOTAL SUGAR; MCCORMICK POTATOES PLANTED IN WET SAWDUST IN GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	TOTAL SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 14	.....	0.663	0.667	0.665
December 14	20° C. to 22° C.	0.572	0.503	0.538
January 20	22° C. to 17° C.	0.663	0.626	0.645
February 3	20° C. to 23° C.	0.430	0.413	0.408
March 26	21° C. to 23° C.	0.537	0.553	0.545

TABLE IV

TOTAL SUGAR; GREEN MOUNTAIN POTATOES STORED IN VAULT

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	TOTAL SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 28....	..... to 5° C.	1.02	1.19	1.10
December 20	5° C. to 2° C.	1.48	1.76	1.62
January 13.....	2° C. to −1.5° C.	1.94	2.26	2.10
February 13.....	−1.5° C. to − 1° C.	3.84	4.04	3.94

OXIDASE.—The juice from new potatoes shows less ability to accelerate the oxidation of pyrogallol than the juice from tubers at the end of the rest period. The juice from the seed half causes no greater acceleration of this oxidation than that from the stem half, even after sprouting (tables VI–VIII).

TABLE V

ACTION OF GLYCERINE EXTRACT OF POTATO PULP ON SOLUBLE STARCH SOLUTION; MCCORMICK POTATOES STORED IN WET SAWDUST IN THE GREENHOUSE

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	INCREASE IN MILLIGRAMS OF SUGAR IN 24 HOURS AT 40° PER 100 GM. OF POTATO PULP					
		Reducing sugars			Total sugar		
		Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 14 ..	.....	65.0	58.0	61.5	65.6	60.0	62.8
December 14...	20° C. to 22° C.	63.2	56.0	59.5	80.3	72.0	79.0
January 20.....	22° C. to 17° C.	91.0	67.2	79.1	72.1	60.5	66.3
February 3.....	20° C. to 22° C.	84.0	66.4	76.8	78.4	63.9	71.1

NITROGEN.—A general survey of the different combinations of nitrogen in the tuber was made at intervals during the rest period in order to determine if protein hydrolysis or other transformations of the nitrogen-containing substances occur during the rest period as necessary antecedents to sprouting. The following determinations were made: total nitrogen, water-soluble nitrogen, nitrogen coagulated by heat, nitrogen precipitated by tannic and by phosphotungstic acids. It was assumed that the nitrogen coagulated by heat represented the protein nitrogen, and the difference between

TABLE VI

OXIDATION OF PYROGALLOL BY JUICE FROM IMMATURE TUBERS JUST HARVESTED AND BY JUICE FROM TUBERS OF THE SAME VARIETY AT THE END OF THE REST PERIOD, BUT NOT SPROUTING

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY	
		New tuber	Old tuber
Hours			
3	33°8 C.	−0.65	−0.7
22	33.8	−1.55	−1.9
25	34.0	−1.70	−2.2

TABLE VII

OXIDATION OF PYROGALLOL BY JUICE FROM NEW TUBERS AND FROM TUBERS OF THE SAME VARIETY AT THE END OF THE REST PERIOD, BUT NOT SPROUTING

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY		
		New tuber	Old tuber	Old tuber calculated to moisture of new tuber
Hours				
5	34°6 C.	−0.4	−0.6	−0.55
26	34.6	−0.8	−1.15	−1.05
46	34.6	−0.85	−1.35	−1.24
71	34.6	−1.50	−2.00	−1.83
125	34.6	−3.05	−3.4	−3.02

TABLE VIII

OXIDATION OF PYROGALLOL BY JUICE FROM THE SEED AND STEM HALVES, JANUARY 10

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY	
		Seed half	Stem half
Hours			
4	34°6 C.	−0.75	−0.75
6	34.7	−1.45	−1.45
48	34.5	−2.85	−2.90

this and the nitrogen precipitated by tannic acid, the proteose, and the peptone nitrogen. The nitrogen not precipitated by phosphotungstic acid was considered the nitrogen of monoamino acids and their amide derivatives, while the difference between this and the nitrogen not precipitated by the tannic acid was considered the nitrogen of diamino acids and other bases. It is not claimed that the foregoing precipitation method gives absolutely the true proportion of nitrogen in the various forms of binding, but it yielded valuable comparative results under the conditions employed in the determinations.

The nitrogen determinations were made on Green Mountain potatoes harvested on November 4 and planted at once in wet sawdust on the floor of the greenhouse. The temperature variation in the sawdust was slight and the tubers were constantly under favorable growing conditions. Sprouts began to appear on this lot of potatoes on January 19. The set of analyses made on January 18, therefore, shows the nitrogen situation just at the end of the rest period. The tubers used for the last set of analyses bore sprouts from one-eighth to one inch in length. As soon as sprouting began, the tubers were placed in a moist chamber, which was buried in the sawdust; therefore nothing was absorbed by the roots except possibly a little water. Each sample contained 6 tubers with a total weight of about 800 gm. The variation in total weights was not more than 5 gm. The samples were all weighed the day after the potatoes were harvested, and kept separate during the storage in the wet sawdust. This makes possible the calculation of results to percentage of original weight, and thus apparent changes due to loss in dry substance through respiration and also through changes in water content are avoided.

On the above basis of calculation the different forms of nitrogen in the whole tuber showed no general change until the tubers began to sprout. During the rest period the stem half always showed a higher percentage of nitrogen, calculated to percentage of total nitrogen, in the following forms: monoamino acids and amides, diamino acids and other bases, proteoses, and peptone. The seed half contained a slightly higher percentage of both water-soluble and water-insoluble protein nitrogen (tables IX–XI). *The slight*

variation in the relative magnitudes of the above forms of nitrogen during the rest period was no greater than would be expected in different biological samples; in most cases it was well within the experimental error.

TABLE IX

NITROGEN OF THE WHOLE TUBER CALCULATED TO PERCENTAGE OF TOTAL WEIGHT OF EACH SAMPLE ON NOVEMBER 8; TUBERS SPROUTING ON FEBRUARY 12

Date of sampling	Total nitrogen	Water-soluble nitrogen	Nitrogen not coagulated by heat	Nitrogen not precipitated by tannic acid	Nitrogen not precipitated by phosphotungstic acid
November 10...	0.430	0.365	0.232	0.217	0.205
November 29...	0.432	0.371	0.234	0.222	0.210
December 13...	0.429	0.369	0.225	0.211	0.198
December 27...	0.428	0.367	0.234	0.222	0.213
January 18.....	0.424	0.345	0.229	0.217	0.205
February 12....	0.428	0.381	0.239	0.228	0.223

TABLE X

DISTRIBUTION OF NITROGEN IN PERCENTAGE OF TOTAL NITROGEN; TUBERS SPROUTING ON FEBRUARY 12

Date of analysis	Water-soluble protein nitrogen			Water-insoluble proteim nitrogen			Non-protein nitrogen		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 10.....	31.95	29.98	30.96	15.04	14.98	15.01	53.01	55.04	54.02
November 29.....	32.41	30.77	31.59	14.49	13.75	14.12	53.10	55.48	54.29
December 13.....	32.78	31.68	32.23	15.01	13.71	14.36	50.12	54.61	52.36
December 29.....	30.74	31.18	30.96	15.58	12.87	14.22	53.68	55.95	54.31
January 18.....	32.83	28.40	30.61	14.02	13.82	13.92	53.15	57.78	55.46
February 12.....	32.82	30.62	32.81	11.96	10.68	11.30	55.24	58.70	56.97

TABLE XI

DISTRIBUTION OF NITROGEN IN PERCENTAGE OF TOTAL NITROGEN; TUBERS SPROUTING ON FEBRUARY 12

Date of analysis	Proteose and neptone nitrogen			Nitrogen of diamino acids and other bases			Nitrogen of monoamino acids and amides		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 10.....	2.54	3.51	3.02	2.33	3.28	2.80	48.14	48.25	48.19
November 29.....	2.49	3.50	3.00	2.32	2.79	2.56	48.29	49.19	48.72
December 13.....	2.99	3.31	3.15	2.11	3.40	2.75	47.11	47.90	47.51
December 29.....	.....	.....	.....	2.08	3.10	2.59	48.42	50.71	49.56
January 18.....	2.10	3.46	2.78	2.04	3.95	2.99	49.01	50.37	49.69
February 12.....	3.71	3.69	3.70	1.11	1.80	1.45	50.43	53.21	51.75

PHOSPHORUS.—Phosphorus is an essential element of two of the most important constituents of the cell, the nucleoproteins and the lipoids. Protein, lipoid, and extractive phosphorus were determined at intervals during the rest period with a view to ascertaining if certain gradual transformations of the phosphorus combinations occur during the rest period in order to render phosphorus available in the proper form for the growing buds.

TABLE XII

EXTRACTIVE; PROTEIN AND LIPOID PHOSPHORUS CALCULATED TO PERCENTAGE OF TOTAL PHOSPHORUS; MCCORMICK POTATOES PLANTED IN WET SAWDUST IN THE GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF SAMPLING	EXTRACTIVE P			PROTEIN P			LIPOID P		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 14	49.1	47.57	48.33	44.34	45.90	45.12	6.65	6.54	6.58
December 14	50.50	46.76	48.63	43.50	47.13	45.31	6.003	6.099	6.006
January 20	50.20	44.90	47.55	42.88	48.36	45.62	6.915	6.763	6.840
February 3	50.81	44.60	47.70	41.14	48.11	44.62	8.056	7.29	7.073
March 26	52.83	50.08	51.45	37.99	41.99	39.99	9.198	7.93	8.514

TABLE XIII

INORGANIC PHOSPHORUS IN PERCENTAGE OF TOTAL PHOSPHORUS; GREEN MOUNTAIN POTATOES STORED IN WET SAWDUST IN THE GREENHOUSE; TUBERS SPROUTING ON FEBRUARY 12

DATE OF ANALYSIS	INORGANIC PHOSPHORUS		
	Seed end	Stem end	Whole tuber
November 29	34.23	33.90	34.06
December 13	35.75	34.59	35.17
December 27	33.22	33.08	33.15
January 18	32.71	34.11	33.41
February 12	32.00	34.30	33.15

Calculated to percentage of total phsophorus, it was found that the percentage of extractive phsophorus was consistently higher in the seed end than in the stem end; the percentage of protein phosphorus, on the other hand, was always less in the seed end. *The percentages of all the phosphorus combinations were practically constant throughout the rest period* (tables XII and XIII).

Physiological

Immature potatoes have a thin, slightly suberized skin, which is quite permeable to both water and gases. As the tubers mature, the skin becomes more suberized and more adherent to the underlying tissue. The rapidity and degree of suberization, however, is greatly influenced by moisture; dry conditions favor the process, while moisture retards it. As the skin becomes suberized, its permeability to water and gases is greatly reduced. It occurred to the writer that the skin may very soon become a sufficient barrier between the internal tissues and the external agents to check growth in the buds. This might be due to an external agent becoming a limiting factor in the completion of the growth mechanism in the new tuber, or in the growth itself. The following experiments were planned to test this hypothesis:

The fall crop of McCormick potatoes furnished material for the experiments here recorded, except where otherwise noted. All the experiments, however, were repeatedly confirmed with the summer crop of both Green Mountain and Rehobeth potatoes. The regular mature crop of the McCormick potatoes was harvested on November 4. Tubers were at once planted in the greenhouse in soil, sawdust and sphagnum, but in no case did sprouting occur until January 18; general sprouting did not begin until February. These results were confirmed by similar plantings of McCormick potatoes in 3 successive years. The rest period of McCormick potatoes under natural planting conditions, therefore, is about 90 days from the time the mature crop is harvested at this station. If they are harvested earlier, the rest period is much longer. Immature tubers harvested on September 20, and immediately planted in the greenhouse, did not sprout until February 2.

EFFECT OF REMOVING THE SKIN.—Simply removing the skin from potato tubers at any stage of the rest period will bring about sprouting within 10 days, if favorable external conditions prevail. A number of methods were employed in order to supply the most favorable conditions for sprouting; but the best among those tried consisted in planting the stem ends in wet soil or sawdust and covering the seed ends with 2 or 3 inches of excelsior, kept constantly wet. This method exposes the terminal buds to the maximum partial oxygen

pressure of the atmosphere. The mere greening of the tubers in the subdued light affords considerable protection against decay. Under the foregoing condition the degree of previous corking of the skin greatly influenced the time before the tubers with the skin intact began to sprout.

Tables XIV–XVI record typical experiments which show the facts given above. The temperature of each pot was recorded

TABLE XIV

EFFECT OF REMOVING THE SKIN; IMMATURE TUBERS HARVESTED ON SEPTEMBER 20 AND PLANTED THE SAME DAY; 12 TUBERS IN EACH LOT

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER						AVERAGE LENGTH OF SPROUTS AFTER 35 DAYS
			10 days	20 days	35 days	85 days	110 days	135 days	
Soil.....	Soil	On	0	0	0	0	23	70	0
Soil.....	Excelsior	On	54	62	77	77	.....	85	4 mm.
Soil.....	Excelsior	Off	38	100	100	100	100	100	20 mm.
Sawdust..	Sawdust	On	0	0	0	0	0	42	0
Sawdust..	Sawdust	Off	75	75	100	100	100	100	5 mm.
Sawdust..	Sawdust	Off	57	85	100	100	100	100	10 mm.

TABLE XV

EFFECT OF REMOVING THE SKIN; MATURE TUBERS HARVESTED ON OCTOBER 28 AND PLANTED ON OCTOBER 31; MEDIUM CORKED SKINS; 12 TUBERS IN EACH LOT

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER			
			10 days	20 days	35 days	95 days
Soil.....	Soil	On	0	0	0	16
Soil.....	Excelsior	On	0	0	20	100
Soil.....	Soil	Removed	0	0	25	82
Soil.....	Excelsior	Removed	25	83	100	100

TABLE XVI

EFFECT OF REMOVING THE SKIN; MATURE TUBERS HARVESTED ON NOVEMBER 4 AND PLANTED ON NOVEMBER 22; HEAVILY CORKED SKINS

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER			
			10 days	20 days	45 days	72 days
Sawdust.....	Excelsior	On	0	0	0	43
Sawdust.....	Excelsior	Removed	50	100	100	100

twice daily. The bulb of the thermometer was placed on a level with the terminal bud of the tubers. The variation among the different pots was always less than a degree. The average temperature one inch below the surface of the soil in pot 1, table XIV, was 18°.5 C. for the 135 days.

The elimination of the rest period by removing the skin is not due to water absorption from the exterior, as tubers with the skins removed will sprout even in dry storage much earlier than those with skins intact; see table XVII.

TABLE XVII

EFFECT OF REMOVING THE SKINS AND STORING IN A DRY PLACE; MATURE TUBERS HARVESTED ON NOVEMBER 4; SKINS REMOVED ON NOVEMBER 7 AND PLACED IN MOIST CHAMBER 4 DAYS, THEN IN PAPER SACKS; STORED IN LABORATORY CUPBOARD

SKINS	PERCENTAGE SPROUTED AFTER		
	45 days	64 days	85 days
On	0	30	100
Removed	80	100	100

EFFECT OF CUTTING THE TUBERS IN HALF.—All the experiments so far reported were conducted with whole tubers, in which case sprouting always began first from the eyes on the seed end. The reverse is true, however, when the tubers are cut in half transversely to the long axis, separating the seed from the stem end. This applies only to tubers forced to sprout during the natural rest period. At the end of the rest period there seems to be little difference, in respect to time of sprouting, between the eyes on the seed and stem ends if the tubers are cut in half.

On October 31 the skin was removed from tubers harvested on October 28. They were then cut in half. The halves were stood upright on wet soil and covered with wet excelsior. On November 15 all the stem halves bore sprouts from eyes located near the cut surface.

Two lots of 4 tubers each were selected from McCormick potatoes harvested on November 4. On November 8 the tubers were cut in 4 pieces in the manner shown in fig. 1. In addition the skins

FIG. 1.—Effect of cutting tubers in half; skins removed; stem ends at bottom, bud ends at top; tubers harvested November 4; experiment started November 8; photographed December 5.

were removed from the pieces in one lot; both lots were then placed on wet soil in pots and covered with excelsior, which was kept constantly wet. Within 10 days all the stem pieces with skins removed bore sprouts from eyes near the cut surface. The stem pieces with skins on began to sprout on the 20th day. Figs. 1 and 2 show the growth of sprouts on the 24th day. It will be seen from this experiment that even with the skin intact the buds near a cut surface begin to sprout much earlier than normally, provided the exposed surface is kept moist and suberization thereby retarded. The buds on the pieces with skins removed not only sprouted still earlier, but the sprouts also grew much faster.

On November 13, tubers harvested on November 4 were cut in half transversely to the long axis. The stem halves were then divided into two lots of 5 each. The cut surfaces of one lot were immediately dipped into warm paraffin. When the paraffin cooled, forming a thin layer over the surface, both lots were placed in paper sacks and stored in a dark, dry laboratory closet. On December 18 all of the paraffined halves bore sprouts from buds near the cut surface. The surface underneath the paraffin was still moist and the cell walls very little suberized. The paraffin in drying cracked from the edges sufficiently to allow free access of air. The cut surfaces of those not dipped in paraffin were dry and heavily corked; these did not begin to sprout until a month later. It seems very probable that the surfaces kept moist by paraffin and not allowed to suberize admitted something to the near-by buds which was not so freely admitted through the heavily corked surfaces. It could not be water, as the sprouting occurred in a dry atmosphere. The other alternative is oxygen. The earlier sprouting in the case of the paraffined pieces was not the result of heat applied to the cut surface by the warm paraffin; this was proved when the experiment was repeated, using a third lot which was dipped in paraffin, the paraffin being removed as soon as cold. This lot sprouted no earlier than the one not treated.

EFFECT OF LIGHT.—Planting tubers with the stem ends in soil and covering the seed ends with wet excelsior exposes the latter to subdued light; it is sufficient, however, to induce rather rapid greening of the exposed part of the tuber. It soon became evident by

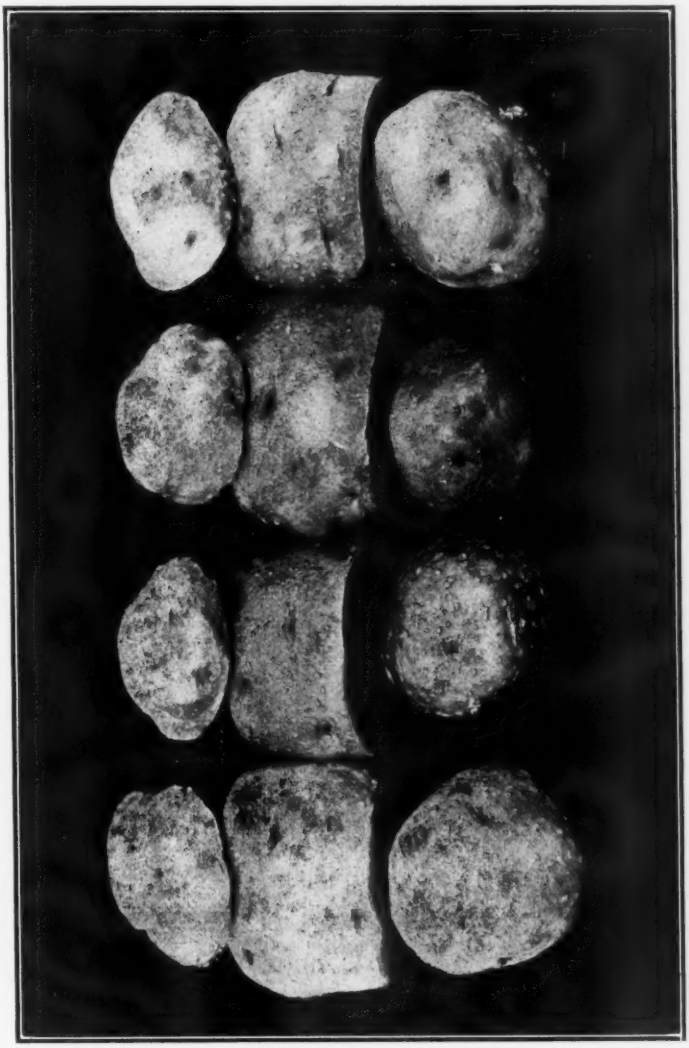


FIG. 2.—Effect of cutting tubers in half; skins not removed; check on fig. 1; also shows early sprouting from buds on the stem ends located near the cut surface.

the use of this method for sprouting tubers that light exercises an influence on the growth processes in the buds. Immature tubers with slightly suberized skins produce sprouts under the influence of subdued light and moisture almost as quickly as they do with the skins removed; see tables XIV and XVIII.

TABLE XVIII

EFFECT OF SUBDUED LIGHT ON IMMATURE TUBERS WITH SLIGHTLY SUBERIZED SKINS; 8 TUBERS IN EACH LOT

SEED HALVES COVERED WITH	PERCENTAGE SPROUTED AFTER			
	20 days	30 days	40 days	50 days
Excelsior	43	43	51	100
Excelsior and black cloth	0	0	14	43

The foregoing light effect is entirely balanced when the skins are removed; for sprouting occurs just as early in the dark as in subdued light, other conditions being comparable (table XIX).

TABLE XIX

EFFECT OF LIGHT WHEN THE SKINS ARE REMOVED; 12 TUBERS IN EACH LOT HARVESTED ON OCTOBER 28 AND PLANTED ON OCTOBER 31

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	PERCENTAGE SPROUTED AFTER		
		10 days	20 days	35 days
Soil	Excelsior and black cloth	25	83	83
Soil	Excelsior	25	83	100

Four lots of 5 tubers each were chosen from immature McCormick potatoes harvested on September 20 and immediately planted in pots with the stem ends in the soil. The projecting seed ends of the 4 lots were covered as follows: (1) a double-walled bell glass filled with a solution of ammoniacal copper sulphate; (2) a similar bell glass filled with a nearly saturated solution of potassium dichromate; (3) a clear bell glass; (4) a black-walled bell glass. By means of bent tubes the air under the bells was in free communication with that on the outside. Through these tubes water was added daily to each pot in sufficient amounts to maintain under the

bells a nearly saturated atmosphere. Equal quantities were added to each pot. Thermometers were placed inside the bell glasses and the temperatures recorded early in the morning and in the afternoon. The temperatures ran practically the same under all the bells, except the clear one, which showed an average of 1–2° higher than the others (table XX).

TABLE XX

EFFECT OF LIGHT ON SPROUTING; IMMATURE TUBERS WITH SLIGHTLY SUBERIZED SKINS

STEM ENDS IN	SEED ENDS COVERED WITH	PERCENTAGE SPROUTED AFTER			AVERAGE LENGTH OF SPROUTS AFTER 35 DAYS
		10 days	20 days	35 days	
Soil	Clear bell	100	100	100	8 mm.
Soil	Red bell	20	100	100	5
Soil	Blue bell	0	0	20	1
Soil	Black bell	0	0	0	0

The experiment recorded in table XX shows not only the stimulating effect of light on the growth processes in the buds, but also suggests that this effect is due to a greater oxygenation of the tissues by photosynthesis. The chief evidence for this conclusion lies in the fact that the tubers under the blue bell glass sprouted very little earlier than those under the black bell glass. Although the tubers under the blue bell glass soon became green, little photosynthesis would be expected, on account of the slight energy for this process in the actinic rays. Subdued light does not hasten the sprouting of the tubers with well suberized skins; the effect is rather one of slight retardation. The rest period of tubers with heavily suberized skins may be considerably extended by thorough greening in full light.

EFFECT OF HYDROGEN PEROXIDE.—If the skin is in any degree permeable to hydrogen peroxide, the abundance of catalase in potato tubers would decompose it, liberating free oxygen. Earlier sprouting would then be expected if oxygen is a limiting factor for growth under normal conditions. Experiments to test this hypothesis were conducted as follows: Tubers were wrapped in cotton saturated with dioxygen, then stored in moist chambers, which were buried in wet sawdust underneath a greenhouse bench.

Untreated tubers were planted in the sawdust just outside of the moist chambers. The sawdust was kept constantly wet (table XXI).

TABLE XXI

EFFECT OF WRAPPING TUBERS IN COTTON SATURATED WITH HYDROGEN PEROXIDE; 6 TUBERS IN EACH LOT

Cotton saturated with	100 per cent sprouted after
3 per cent dioxygen	28 days
100 per cent dioxygen	43
Untreated	76

Table XXI is typical of a number of experiments that were performed with new potatoes. Such treatment failed, however, to shorten the rest period of tubers with heavily suberized skins; this alteration of the skin doubtless renders it impermeable to hydrogen peroxide. Numerous attempts to control these experiments by wrapping tubers in cotton saturated with distilled water usually failed on account of the decay of the tubers. It may be assumed, however, that the partial oxygen pressure in the wet sawdust would not be less than that under saturated cotton.

RESPIRATION.—It has been shown that the rest period of potato tubers can be either entirely eliminated or greatly shortened by means which would seem to facilitate the oxygenation of the internal tissues. That a great increase in oxygen absorption actually occurs is proved conclusively by the effect of the various treatments on respiration, the rate of which was determined by the amount of carbon dioxide expired from the tubers. Ten tubers, with a total weight of about 1500 gm., were used for each determination, which was allowed to run 24 hours at room temperature. The control determinations were made at the same time and under exactly the same conditions.

The amount of carbon dioxide expired from new potatoes with thin, slightly suberized skins is much greater than that from the same tubers after the skins have become well corked and adherent to the underlying tissues. When the latter character of the skin is attained, the rate of respiration under uniform conditions remains fairly constant until the beginning of sprouting. Table XXII is a typical experiment which will suffice to show this fact. Immature

Rehobeth potatoes grown in the greenhouse and harvested on January 21 furnished the material for this experiment. The tubers were stored in a dry, warm laboratory drawer.

TABLE XXII

RELATION OF RESPIRATION TO SKIN SUBERIZATION

Date of determination	Thermograph average of room temperature	Milligrams of CO_2 per kilo per hour
January 21	20°.3 C.	24.7
January 26	20.0	22.4
February 2	21.1	12.3
February 26	22.2	8.26
March 3	22.0	8.08

Removal of the skin from mature tubers more than doubles the rate of respiration; it falls again with the formation of a new thoroughly corked skin to that of unpared tubers (table XXIII).

TABLE XXIII

EFFECT OF REMOVING THE SKIN

DATE OF DETERMINATION	MILLIGRAMS OF CO_2 PER KILO PER HOUR		RATIO
	Untreated	Skins removed	
November 7	14.94	34.7	1:2.32
November 10	12.02	26.37	1:2.19
December 2	10.9	11.8	1:1.08

MCCALLUM (**17a**) found ethyl bromide especially effective in forcing the resting buds of potatoes into growth. The writer studied the effect of this treatment on respiration in the tuber and found that it has about the same accelerating effect as removing the skin (table XXIV).

It has been shown that the rest period can be shortened by wrapping the new tuber in cotton saturated with hydrogen peroxide. This treatment also accelerates the rate of respiration in new tubers (table XXV).

It is obvious that the elimination or abbreviation of the rest period under the conditions employed in this work is correlated with

greater oxygen absorption. It does not necessarily follow, however, that sprouting was brought about by the greater energy release resulting from the increased respiration. The facts seem to indicate that this was not the case. It is more probable that under normal conditions the skin becomes suberized before the completion of

TABLE XXIV

EFFECT OF TREATMENT WITH ETHYL BROMIDE GAS

DATE OF DETERMINATION	MILLIGRAMS OF CO_2 PER KILO PER HOUR		RATIO
	Untreated	Ethyl bromide gas—30 minutes	
January 18	7.26	28.54	1:3.93
January 21	7.08	27.85	1:3.83

some growth mechanism requiring oxygen. The rate of oxygen diffusion through the suberized skin then determines the time, the natural rest period, required for the perfection of the growth mechanism.

TABLE XXV

EFFECT OF TEATMENT WITH HYDROGEN PEROXIDE; NEW TUBERS

EXPERIMENT	MILLIGRAMS OF CO_2 PER KILO PER HOUR	
	Lot 1	Lot 2
1	Untreated 22.41	Untreated 24.65
2	Wrapped in sterilized cotton saturated with sterilized water 22.9	Wrapped in cotton saturated with 50 per cent dioxygen 39.3

The rôle of oxygen in plant physiological processes is very complex and at the present time quite obscure. However, several cases are noted in the literature which show the wide range of oxygen influence in growth processes. IVANOFF (**11**) claims that oxygen is necessary for the transformation of zymogen into zymase. ZALESKI (**32**) has shown that protein synthesis is influenced by oxygen, etc.

Summary and conclusions

Under normal planting conditions potato tubers will not sprout for several weeks after harvest. During this rest period certain changes must occur in the chemical or physical situation of the buds or their immediate environment which are essential to the release of the growth processes. These changes are spoken of as "after-ripening," using the term in its broadest sense.

The carbohydrate transformations during the rest period are dependent entirely upon changing temperature.

Active diastase and invertase are present at all stages of the rest period, but show no increase under normal growing conditions until the tubers begin to sprout.

The juice from tubers at the end of the rest period causes a greater acceleration of the oxidation of pyrogallol than the juice from new immature tubers of the same variety. The seed and stem halves show no difference in the ability to oxidize pyrogallol even after sprouting from the seed end.

After-ripening does not involve protein hydrolysis. There is no change during the rest period in the relative magnitudes of the following forms of nitrogen: proteose and peptone; diamino acids and other bases; monoamino acids and amides.

Protein, lipoid, organic extractive, and inorganic phosphorus calculated to percentage of total phosphorus, each remains constant up to the time of sprouting.

The magnitudes of the substance studied are not all identical in the seed and stem halves. The relative composition, however, remains practically constant during the rest period in spite of the fact that sprouting begins much earlier on the seed end.

Metabolic changes involving the above forms of nitrogen and phosphorus begin rather suddenly and are concurrent with sprouting. The same is true of diastase.

Drying causes rapid suberization of new skin and exposed surfaces.

Suberization greatly reduces the permeability of the skin to water and gases.

Potatoes may be sprouted at any time during the rest period by simply removing the skins and supplying the tubers with favorable

growing conditions, which include in this case the maximum partial oxygen pressure of the atmosphere. The elimination of the rest period by this means is not due to water absorption from the exterior, as tubers with skins removed will sprout, even in dry storage, much earlier than those with skins intact.

If tubers are cut in half transversely or cut into half-inch slices, the buds on the stem half located near the exposed surface will sprout much earlier than normally, provided suberization of the surface cells is prevented. This may be accomplished by laying them on wet soil, or, better still, sawdust, and covering with wet excelsior. Sprouting in this case also was not due to water absorption, because the rest period of these buds may be greatly shortened in dry storage if drying of the exposed surface is prevented by covering it with a thin layer of paraffin.

The earliest sprouting occurred when the skins were removed and the tubers also cut in the manner described above.

Subdued light stimulates growth in buds on new tubers with slightly suberized skins. The evidence at hand makes it highly probable that this effect is due to greater oxygenation of the tissues by photosynthesis. The light influence entirely disappears when the skin is removed. Subdued light does not stimulate growth in the buds on tubers with highly suberized skins; the effect is rather one of retardation.

The rest period of new potatoes may be shortened by wrapping the tubers in cotton saturated with hydrogen peroxide. The abundance of catalase in potato tuber decomposes the hydrogen peroxide diffusing through the thin skin, liberating free oxygen. This treatment had no effect on old tubers on account of the impermeability of the heavily suberized skin to the hydrogen peroxide.

All the foregoing treatments greatly accelerate the rate of respiration. It may be safely concluded, therefore, that the elimination or abbreviation of the rest period under the conditions employed in this work is correlated with increased oxygen absorption.

The rest period of the potato tubers is not firmly fixed and hereditary. It is not of internal origin due to autogenic metabolic changes, as it can be entirely eliminated by means which maintain a proper adjustment between the bud tissues and external agents,

chiefly oxygen. In nature the oxygen supply to the internal tissues is regulated by skin characters, which are greatly influenced by moisture regulations.

MARYLAND AGRICULTURAL EXPERIMENT STATION
COLLEGE PARK, MD.

LITERATURE CITED

1. ASKENASY, E., Über die jährliche Periode der Knospen. Bot. Zeit. **37**: 366, 367. 1877.
2. ———, See PFEFFER, Pflanzenphysiologie **11**:260. 1904.
3. APPLEMAN, CHARLES, Physiological behavior of enzymes and carbohydrate transformations in after-ripening of the potato tuber. BOT. GAZ. **52**:306–315. 1911.
4. BUTKEWITSCH, W. L., Zur Frage über die Umwandlung der Stärke in den Pflanzen und über den Nachweiss der amylolytischen Enzyme. Biochem. Zeit. **10**:314. 1908.
5. CROCKER, WILLIAM, Rôle of seed coats in delayed germination. BOT. GAZ. **42**:265–291. 1906.
6. CHRISTENSEN, P., Chemical researches of bulbs in the later phases of the rest period. Bull. Acad. Roy. Dänemark (Copenhagen), p. 44. 1908.
7. EULER, H., Grundlagen und Ergebnisse der Pflanzenchemie. Braunschweig. 1909.
8. FISHER, ALFRED, Beiträge zur Physiol. der Holzewachse. Jahrb. Wiss. Bot. **22**:73. 1891.
9. GRÜSS, J., Enzymewirkungen aus Wundrand der Kartoffelknolle. Zeit. Pflanzenkrank. **17**:69–70. 1907.
10. HOWARD, W. L., An experimental study of the rest period in plants. The winter rest. Research Bull. 1, Agric. Exper. Sta. Univ. Mo. 1910.
11. IVANOFF, L., Über die sogenannte Atmung der zerriebenen Samen. Ber. Deutsch. Bot. Gesells. **29**:563–570. 1911.
12. JESENKO, E., Einige neue Verfahren der Ruheperiode des Holzewachse abzukurzen. Ber. Deutsch. Bot. Gesells. **29**:273–283. 1911.
13. JOST, L., Wachstum wahrend der Ruheperiode. Bot. Zeit. **51**:100. 1893.
14. JOHANNSEN, W., Das ather Verfahren beim Fruhtreiben. Jena. 1906.
15. KLEBS, G., Über die Rhymik in der Entwicklung der Pflanzen. Sitz. Heidelb. Akad. Wiss. **23**:1–84. 1911.
16. ———, Die periodischen Erscheinungen tropischer Pflanzen. Biol. Centralbl. **32**:257–285. 1912.
17. LAKON, GEORGE I., Die Beeinflussung der Winterruhe der Holzgewachse durch die Nahrsalze. Zeitschr. Bot. **4**:561–582. 1912.
17a. MCCALLUM, W. B., Twentieth Ann. Rep. Univ. Ariz. Agric. Exper. Sta. p. 586. 1909.

18. MOLISCH, H., Über ein einfaches verfahren Pflanzen zu treiben (Warmbad Methode). Sitz. Kais. Akad. Wiss. **117**:87–117. 1908.
19. MÜLLER-THURGAU, H., Beiträge zur Erklärung der Ruheperiode der Pflanzen. Landw. Jahresber. **11**:751–828. 1882.
20. ———, Beiträge zur Erklärung der Ruheperiode der Pflanzen. Landw. Jahrb. **14**:859–863. 1885.
21. MÜLLER-THURGAU, H., und SCHNEIDER-ORELLI, O., Beiträge zur Kenntniss der Lebensvorgänge in ruhender Pflanzen. Über den Einfluss der Vorerwarmens und einiger anderer Fakloren. Flora **101**:309–372. 1910.
22. ———, Beiträge zur Kenntniss der Lebensvorgänge in ruhenden Pflanzenteilen. Flora **104**:386–446. 1912.
23. SCHMID, B., Über die Ruheperiode der Kartoffelknollen. Ber. Deutsch. Bot. Gesells. **19**:76. 1901.
24. SIMON, S., Untersuchungen über das Verhalten einiger Wachstumsfunktionen, sowie der Atmungstätigkeit der Laubholzer während der Ruheperiode. Jahrb. Wiss. Bot. **43**:1–35. 1906.
25. SCHIMPER, A. F. W., Pflanzengeographie auf physiologischer Grundlage. Jena. 1898.
26. URSPRUND, A., Zur Periodicität des Dichenwachstums in den Tropen. Bot. Zeit. **62**:189–210. 1904.
27. IRAKLIONOW, P. P., Über den Einfluss des Warmbads auf die Atmung und Keimung des ruhenden Pflanzen. Jahrb. Wiss. Bot. **51**:515–539. 1912.
28. VOLKENS, G., Der Laubwechsel tropischer Bäume. Gartenflora **52**:591–598. 1903.
29. ———, Laubfall und Lauberneurung in den Tropen. Berlin. 1912.
30. WEBER, F., Über die Abkurzung der Ruheperiode der Holzgewächse durch Verletzung der Knospen beziehungsweise Injektion dieselbe mit Wasser. Kais. Akad. Wien **10**:182–183. 1911.
31. WISMEWSKI, P., Beiträge zur Kenntnis der Keimung der Winterknospen der Wasserpflanzen. Bull. Inter. Acad. Sci. Cracovie B. pp. 1045–1060. 1912.
32. ZALESKI, W., Zur Kenntnis der Stoffwechselprocess in reifenden Samen. Beih. Bot. Centralbl. **27**:63–82. 1911.

DECAY AND SOIL TOXINS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 213

GEORGE B. RIGG

The decomposition products of a specific plant organ and their effects on the growth of other plants furnished the point of attack for the work on toxicity reported in this paper. The material used was the rhizomes of *Nymphaea advena* Ait. and *N. polysepala* Greene. This material was obtained at intervals from July 1912 to October 1915, at various places in the vicinity of Chicago, Illinois, and Seattle, Washington.

Review of literature

RELATED WORK ON TOXICITY

The organic constituents of soils have been under investigation by workers in the United States Bureau of Soils for 10 years. LIVINGSTON (**12, 13**) found toxic substances, probably organic, in an unproductive soil. SCHREINER (**21**) and his co-workers have isolated from soils more than 25 organic compounds differing widely in chemical character. Some of these (for example, dihydroxystearic acid) have proved harmful to growing plants; some (for example, nucleic acid) have been found beneficial; and some have not been shown to have any effect on the growth of plants. BOTTOMLY (**3**) has found that certain aerobic organisms grow well in peat and form from it compounds that are beneficial to the growth of plants. He suggests that very small amounts of accessory organic substances may be necessary for the growth of plants.

Humic acid has been much discussed as a possible factor in plant growth. Not only the effects of this so-called humic acid, but also the constitution and nature of the substance are in doubt. SCHREINER (**21**) regards it as a mixture of substances. WIELER (**29**) takes the view that humic acids in soils are inorganic acids resulting, for example, from the chemical decomposition of salts.

BAUMAN and GULLY (**2**) have suggested that the acidity of bog water is due to the fact that the cell colloids of the disintegrating

plant tissues retain chiefly the basic ions of the salts dissolved in the cell contents of the plant tissues before they began to decay, thus freeing the acid ions. SKENE (**24**) has found that various species of *Sphagnum* thrive best in acid solution because mineral solutions, although usually physiologically harmless, may be ecologically harmful.

Work by LIVINGSTON (**14**), DACHNOWSKI (**6, 7**), the writer (**19**), and others (**20**), indicates that the inhibition from sphagnum bogs of plants other than bog xerophytes is not due to acidity, or to low surface tension, or to high osmotic pressure of the soil solution, but is due in part to the presence of toxic substance or substances in the soil solution.

Many workers (**8, 9, 15–18, 25–27**) have found that cultivated crops and plants grown in cultures have a favorable or an unfavorable influence on other plants growing in the same substratum either at the same time or subsequently. Food supply and toxins have been suggested as means through which this influence may be exerted. CZAPEK (**5**) finds that the roots of plants are injured when the surface tension of the bathing solution is lower than 0.66.

SHERFF (**23**) found in Skokie marsh near Chicago that where the rhizomes of *Sagittaria latifolia* had penetrated the decaying rhizomes of *Nymphaea advena*, they themselves had begun to decay.

STERILE CULTURES OF SEED PLANTS

More or less success has been attained by various workers in attempts to grow seed plants under sterile conditions. HARRISON and BARLOW (**11**) tried sterilization by dry heat, moist heat, sulphuric acid, calcium hydrate, formaldehyde, and mercuric chloride, and abandoned all of these means. They succeeded in getting sterile cultures of certain legumes by treating the unopened pods with mercuric chloride, opening them with flamed forceps, and transferring the seeds to a very small quantity of boiling water in sterile test tubes.

WILSON and HARDING (**30**) tried alcohol, formaldehyde, and mercuric chloride as a means of sterilizing alfalfa seeds, but found that when the seeds were sterile, the germination was very low.

Using a modification of HARRISON and BARLOW'S method, they got alfalfa seedlings which grew in sterile cultures for 4 months.

BROWN (4) found that barley seeds take up water from a fairly strong solution of sulphuric acid and remain uninjured. SCHROEDER (22) found silver nitrate to be a good means of sterilizing wheat. He found that hand-picked wheat endured soaking in a 5 per cent solution of this substance for 24 hours without injury, and for 72 hours with but slight injury. Threshed wheat, however, because of the rupture of semipermeable membranes by the machinery, would not stand such prolonged treatment. ARCHICHOWSKY (1) got a large percentage of sterile cultures of seed plants by the use of formaldehyde and other antiseptic agents on peas, pumpkins, and other seeds.

Solutions and preparations

Both of the species of *Nymphaea* used produce branched rhizomes 3–15 cm. thick and sometimes reaching as great a length as 3 m., although they are more commonly 1 m. or less. The older portions of these rhizomes decay. SHERFF (23) found these rhizomes decaying to within a short distance of the growing apex. The writer has found the decay only in older portions of the rhizome. Sound pieces of the rhizome were collected and the following solutions were made up quantitatively, each solution having a volume of 1600 cc. and containing the solutes obtained by the methods described from 1000 gm. of fresh rhizome. An average of 3 tests on the water contents of the fresh rhizome gives 88 per cent of water. The tests were made by cutting 500 gm. of the fresh material into small pieces and drying it at 105° C.

Solution 1A.—This was the liquid resulting from the decay of 1000 gm. of fresh *Nymphaea* rhizome in redistilled water, freed from solid matter by filtering through cheesecloth, and diluted to 1600 cc. with redistilled water. Molds continued to grow on the surface of this solution. It was amber colored.

Solution 1B.—This was the solution remaining after a duplicate of 1A had been extracted by shaking with an equal volume of ether in a separatory funnel. The ether that dissolved in the water solution was removed by heating to 40° C. and subjecting to

a suction of 2 cm. of mercury with an aspirator. Molds continued to grow on the surface of this solution. It had a slightly darker color than 1A.

Solution 1C.—The ether used in extracting 1B was allowed to evaporate spontaneously. The solid remaining was reddish-brown, and only partially soluble in water. This solution consists of 1600 cc. of redistilled water, and all the residue from the ether extraction that would dissolve in that quantity of water at 40° C. No molds grew on this solution, and no scum or turbidity or other evidence of bacterial activity appeared.

Preparation 1D.—This was the solid remaining from filtering 1A. This solid was ground with an equal volume of sand.

Solution 2A.—This was the distillate under reduced pressure (2 cm. pressure), at 40° C., of the liquid and solid products of the decay of 1000 gm. of *Nymphaea* rhizome in redistilled water. This was a clear liquid having the appearance of water. No molds grew upon it, and it showed no evidence of bacterial activity.

Solution 2B.—The solid remaining from the distillation of 2A was dried in an oven at 30° C. and then ground in a mortar. It was then black powder. This was extracted in a Soxhlet apparatus with ether. When the ether was allowed to evaporate spontaneously, a sticky, semi-solid, reddish-yellow substance remained. This was only partially soluble in water. This solution represents 1600 cc. of redistilled water, with all of the ether extract that would dissolve in it at 40° C. It had a light reddish-yellow color. No molds grew upon it, and no evidence of bacterial activity appeared.

Solution 2C.—The solid remaining from the extract of 2B was exposed to air until all odor of ether had disappeared. It was then extracted for 2 hours with 1600 cc. of redistilled water. It was perfectly clear. No molds or evidence of bacterial growth appeared.

Preparation 2D.—This represents the solid remaining from the extract of 2C, ground in a mortar with an equal volume of sand to form a soil.

Solution 3A.—One kg. of fresh *Nymphaea* rhizome was cut into pieces, ground in a meat grinder, and the juice pressed out in a fruit press. The solid remaining was extracted with ether, and

afterward exposed to the air until the odor of ether disappeared. It was then allowed to decay in redistilled water. The liquid resulting from this decay was strained through cheesecloth and diluted with redistilled water to 1600 cc.

Solution 3B.—The juice squeezed out in preparing solution 4A was extracted by shaking with an equal volume of ether in a separatory funnel, which was then freed from ether by heating to 40° C., and subjecting to suction (2 cm. of mercury) by means of an aspirator for 24 hours. This, when diluted 1600 cc., constituted solution 3B.

Solution 3C.—The ether used in extracting 3A was combined with that used in extracting 3B. This was allowed to evaporate spontaneously and as much of the residue as possible was taken up in 1600 cc. of redistilled water at 40° C.

Preparation 3D.—The solid matter remaining on the cheesecloth in the preparation of 3A was air-dried and ground with an equal volume of sand to form a soil.

Solution 4A.—This was the water extract of the fresh rhizome made under sterile conditions. The rhizome was cut into small pieces and placed in flasks with water. These flasks were stoppered with cotton and sterilized in an autoclave. This solution stood sterile for 11 months before its toxicity was tested.

Solution 5A.—This was the water solution of the ash from the fresh rhizome.

All sand used in the previous preparations and in the following experiments was either no. $2\frac{1}{2}$ quartz or "Ottawa test." In all cases it was washed in 10 per cent HCl, freed from acid by washing in running water, and finally rinsed with redistilled water.

The "Knop's solution" had the following composition: 1 part KNO_3; 1 part K_2HPO_4; 1 part $Mg\,SO_4$; 4 parts $Ca(NO_3)_2$. This was made up to 0.1 per cent.

Where "tap water" is mentioned, the water used was Chicago city water. Where "Cedar River water" is mentioned, the water used was Seattle city water, which is piped from the river near its origin in a snow-fed lake. Where "Lake Washington water" is mentioned, the water is that supplied from Lake Washington to the botany laboratories at the University of Washington.

Experiments and results

In order to determine the relative toxicity of these various solutions to *Tradescantia*, cuttings of the plant were placed in various dilutions of each solution with redistilled water. In this way the percentage of the solution (that is, the number of cc. diluted to 100 with redistilled water) that would allow the formation of roots but inhibit the production of root hairs was determined.

All of the solutions except 5A were acid to both litmus and phenolphthalein. Their acidity was determined by titrating with N/10 NaOH, using phenolphthalein as an indicator.

Table I gives the toxic limits of these solutions to *Tradescantia* (as previously defined) and their acidity, together with the relative rank of each solution as to toxicity and acidity and the ratios of these.

TABLE I

Solution acidity	Toxicity (per cent)	Rank as to acidity	Rank as to toxicity	Ratio of acidity to toxicity
1A N25/1000	7.5	1	1, 2, or 3	0.27
1B N24/1000	7.5	2	1, 2, or 3	0.25
1C N11/1000	7.5	4	1, 2, or 3	0.12
2A N22/1000	10.0	3	4	0.24
2B N6 /1000	50.0	7	7	0.12
3A N9 /1000	12.5	5	5	0.10
3B N7 /1000	15.0	6	6	0.08
3C N5 /1000	75.0	8	8	0.06

All of the solutions mentioned in table I, except 3B, were neutralized to phenolphthalein with N/10 sodium hydrate, and the effect of both the acid and the neutral solution was tried on *Tradescantia* cuttings. Table II shows the results of the dilutions named on *Tradescantia* cuttings.

TABLE II

Solution	Strength (per cent)	Acid	Neutralized
1A	7.5	Root hairs none	Root hairs normal
1B	5.0	" " slightly stunted	" " "
1C	5.0	" " " "	" " normal
2A	7.5	" " none	" " slightly stunted
2B	60.0	Roots none	Roots 3–10 mm. long
3A	12.5	Root hairs none	Root hairs slightly stunted
3C	10.0	" " slightly stunted	" " normal

The toxicity of the first 4 of these solutions when undiluted was not perceptibly lowered by neutralization with sodium hydrate.

A 20 per cent solution of 1A was shaken in a large flask 5 times a day for 10 minutes each time, for 3 days in order to aerate it, and then filtered through a filter paper. Tests on *Tradescantia* indicated that its toxicity was not decreased by this treatment.

A 20 per cent solution of solution A in tap water was shaken with animal charcoal and filtered. The filtrate was colorless and odorless. The solution before this treatment had an amber color and a foul odor. Cuttings of *Tradescantia* were placed in this. In all cases they grew well and produced good roots with normal or only slightly stunted root hairs.

The animal charcoal with which this solution was shaken was used as a soil for cultures of alfalfa seed. Controls of animal charcoal shaken with tap water were run. The growth in the controls was twice as great as the growth in the cultures.

The toxicity of preparation 2D to *Tradescantia* cuttings was tested by planting the cuttings in the preparation in small flower pots. This preparation was further diluted also by grinding with half its volume of sand. For convenience this further dilution is referred to in table III as preparation 2Dx. Controls were run

TABLE III

Medium	Roots	Plants
2D	None	Dying
2Dx	Normal	Healthy
Potting soil	Normal	Healthy

in potting soil. All of the pots in a set were placed together in a large glass dish, so that they stood in about one-fourth of an inch of tap water and thus were all watered alike. Table III shows the results at the end of 14 days.

Preparation 3D was tried in the same way with similar results. Preparation 1D was tried in the same way with corn and did not prove toxic. Preparations 2D and 3D are not at all toxic to alfalfa.

The toxicity of solutions 1A, 1B, 1C, and 2A to unsterilized corn was tested by planting the seeds in 200 cc. of sand watered with 25 cc. of solution. The cultures were in 500 cc. Erlenmeyer

flasks, stoppered with cotton. Controls watered with Knop's solution were run. All of the solutions tested proved toxic, but 2A was found to be less toxic to corn under these conditions than the others. The toxicity of the solutions was shown in the injury to the root tips, causing a great decrease in the total length of root produced as compared with the controls, and thus eventually killing the plants.

The effect of the 8 solutions listed in table I was tried on unsterilized alfalfa in sand. They were all found extremely toxic, many of the plants dying as they emerged from the sand, and the best of them attaining only one-fifth of the height attained by controls watered with tap water.

It was found by tests that tomato seeds did not germinate at all in solution 1A in sand, while controls in sand watered with tap water grew vigorously.

An attempt was made to secure sterile cultures of corn in 500 cc. Erlenmeyer flasks in order to determine whether the presence of organisms in the cultures was a factor in the toxicity of these cultures. The sterility of the cultures was tested by making bouillon cultures from various portions of the sand and the seeds at the end of the experiment; 65 per cent of the cultures proved sterile.

Three problems were to be solved in working out a method of securing sterile cultures of seed plants: (1) the sterilization of the flasks and contents; (2) the sterilization of the seeds; (3) the transfer of the sterile seeds to the sterile flasks under sterile conditions.

The cotton-stoppered flasks, containing 200 cc. of sand and 20 cc. of the solution, were sterilized for 1 hour at 15 lbs. pressure in the autoclave. A solution of silver nitrate was used as a means of sterilizing the seeds. It was found by experiment that corn would germinate well after treatment for 1 minute with N/300 $AgNO_3$ and subsequent washing with water. As a means of making the transfer of the seeds to the flask, the box previously used by JENSEN (**11a**) was used. When the lid of the box was closed, the operator could thrust his hands into the gloves and work without danger of contaminating the cultures from any source outside of the box. Since the entire top and part of the front of

the box were of glass, all articles inside of the box could be seen readily by the operator.

The entire inside of the box, including the side of the gloves and sleeves exposed to the air of the box, and the outside of all flasks and other articles placed in the box, were treated thoroughly with a 15 per cent solution of glycerine saturated with carbolic acid. All of the cultures prepared were thus more or less exposed to carbolic acid fumes. This solution was applied by means of a sponge attached to a short stick. It was found that flasks and dishes stuck to the paint treated with this mixture, so that a false bottom of glass was placed in the box. The stoppers of the sterilized flasks (including 2 of sterile water) were flamed, and the flasks placed in the box. Four tall stenders with ground glass covers were sterilized in an oven for 5 hours at 120° C., and placed in the box. A bottle of N/300 $AgNo_3$ was also placed in the box, as were also a pair of 10-inch brass forceps and a waste jar containing a little of the glycerine carbolic acid solution. Everything in the box having been treated with the antiseptic solution, the cover of one of the stenders was removed and dry corn placed in the stender and the cover quickly replaced. In selecting corn for this purpose, care had been taken to secure smooth kernels that would offer little opportunity for the lodgment of air bubbles in the dent or elsewhere.

The box now remained closed over night, to allow any organisms in the air to settle to the bottom of the box and be held by the glycerine solution. In the morning the operator thrust his hands into the gloves, poured $AgNO_3$ on the seeds, left it on for 1 minute, poured it off into the waste jar, and then washed the seeds in several changes of sterile water, finally allowing them to soak in it for several hours, and rinsing them again. The tips of the forceps now were washed thoroughly in the sterile water and the transfer of the seeds was made. In some cultures the seeds were thrust down into the sand by means of these forceps, and in some cases they were left on the surface. After 2 or 3 cultures had been made, the forceps were placed in the antiseptic solution for a few minutes and again rinsed in sterile water. After the cultures were prepared, they were removed from the box and placed near a window in the laboratory.

The results showed that solutions 1A, 1B, 1C, and 2A (these were the only ones tried) are just as toxic under these conditions as when organisms were known to be present in abundance. Since 65 per cent of these cultures were shown (by bouillon cultures from them at the close of the experiment) to be sterile, it is evident that the presence of organisms is not a necessary condition of the toxicity of these solutions.

Solutions 1A, 1B, and 1C likewise proved toxic to alfalfa in cultures prepared as previously described for corn. The same precautions for securing sterility were observed, but tests of sterility were not made.

The toxicity of solution 4A to *Tradescantia* cuttings was tested as follows: wide-mouth 50 cc. bottles were filled with dilutions of the following strength: 20, 15, and 10 per cent. Into each of these was placed a cutting of *Tradescantia*. The mouths of the bottles were left open. At the end of 16 days no root hairs had formed on any of these plants and all of the plants were showing signs of death. The controls in Lake Washington water all had abundant root hairs and were in healthy condition.

Other sets of bottles were prepared as previously described, except that the mouths were stoppered with cotton and the bottles containing the liquids were sterilized in the autoclave. The dilutions were as follows: undiluted, 20, 15, and 10 per cent. Each cotton stopper was then displaced just enough to allow a cutting of *Tradescantia* to be placed in the liquid. None of these plants developed normal root hairs. All of them except those in the 10 per cent dilution showed signs of death at the end of 16 days. Turbidity due to the action of organisms was evident in all of these, and molds grew on some of them.

Flasks (500 cc.) were prepared, cotton-stoppered, each containing 180 gm. of sand and 18 cc. of solution. These were autoclaved at 12 lbs. pressure for 1 hour. Solution 4A was used pure and also in the following solutions: 20, 15, and 10 per cent. Controls with tap water were also run. Corn was treated with N/100 silver nitrate for 2 minutes, then with sterile forceps 5 kernels were placed in each flask. At the end of 16 days the growth was noticeably greater in all of the controls than in any of the solutions. All

of the flasks except 2 (one control and one 15 per cent) had molds growing in them. Bouillon and also agar cultures were made from each of these, and the 15 per cent flask proved to be sterile. The other was not sterile. These 2 flasks were kept a week longer and the growth in the control was much better than that in the 15 per cent solution.

The transfers of the corn in these cases were made in the laboratory, without the use of the sterile box just described. It is hoped that by autoclaving the flasks at a higher pressure, and treating the seeds with the silver nitrate for a longer period, a larger number of sterile flasks could be obtained.

Solution 1A was filtered and the fresh filtrate was immediately saturated with ammonium sulphate. The sulphate was added gradually and the solution was shaken after each addition. No precipitate appeared at once, but when it had stood over night a considerable amount of a brownish precipitate was present. Some of the precipitate was at the surface of the liquid, some had settled to the bottom, and some particles were in suspension in the liquid.

The precipitate was filtered off and redissolved in a volume of Cedar River water equal to the original volume of the solution. Both filtrate and precipitate were then dialyzed in dialyzing tubing in running water for 11 days. At the end of this time they showed no precipitate with barium chloride. The filtrate and the precipitate were then tested for toxicity by placing *Tradescantia* cuttings in them. Root hairs were formed in both, but their development was poorer in the solution of the precipitate than in the filtrate.

In preparing solution 5A, 2.83 gm. of ash were obtained from 500 gm. of fresh rhizomes. This is 4.7 per cent of the dry weight. Approximately half of this went into solution when shaken with 800 cc. of Cedar River water at 18° C. The solution was basic to litmus. In all cases tried with 5A and its duplicates the toxicity to *Tradescantia* cuttings was so marked that practically no root hairs developed and the plants soon died. When dilutions were tried it was found that all dilutions down to 10 per cent (10 cc. solution to 90 cc. water) inhibited root hair production; in 5 per cent dilution root hairs developed normally.

A solution was prepared from each of the following substances, by allowing a quantity of it to decay in redistilled water: potato, turnip, rhizome of *Castilia odorata*, and rhizome of *Typha latifolia*. These solutions approximated the strength of solution 1A prepared from *Nymphaea*. They all proved toxic to *Tradescantia* cuttings, but to a less degree than 1A did. Their toxicity was in the order named.

Discussion

It is evident from the data given that even very dilute solutions of the products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings in water cultures and the seedlings of tomato, alfalfa, and corn in sand cultures.

Although the products of the decay of the subterranean parts of other plants proved toxic, the toxicity of the products of the decay of *Nymphaea* rhizomes was considerably greater than that of any other plant parts experimented on. While it is possible that toxicity from decay is rather common, *Nymphaea* seems to merit particular attention in this regard. The dilution of solution 1A that entirely inhibited the formation of root hairs on *Tradescantia* cuttings contained in each cc. the products of the decay of 4.7 mg. of fresh rhizome. Since only 12 per cent of the fresh rhizome is solid matter, the amount of solid whose decay contributed to the solutions in each cc. of the toxic solution was 0.56 mg.

The fact that the solutions listed in table I were all acid, and that their toxicity was largely destroyed by neutralization with sodium hydrate, would seem to suggest acidity as a large factor in the toxicity. The toxicity is not proportional to the acidity as determined by the titration method. It may be proportional, however, to the H ion concentration, or some other factor may be effective. The fact that the toxicity of 1A, 1B, 1C, and 2A when undiluted was not reduced by neutralization with sodium hydrate seems to emphasize further the presence of some other factor. It is possible that the osmotic pressure of such a concentrated solution was high enough to cause injury, although it has been shown elsewhere (**20**) that this is not the cause of the toxicity of the very dilute solutions. Antagonistic action on permeability might also

be a possible factor in the lowering of toxicity on the addition of sodium hydrate.

The fact that the toxicity of solution 1A was not destroyed by the aeration here reported does not necessarily mean that the toxicity cannot be removed by oxidation.

The removal of the toxicity from solution 1A by shaking it with animal charcoal is probably to be explained as an absorption phenomenon.

The only importance attaching to the toxicity of the soils (preparations 1D, 2D, and 3D) is that not all of the water-soluble toxic materials had been washed out of them by the treatments to which they had been subjected.

The products of the decay of *Nymphaea* rhizomes are toxic, not only to *Sagittaria* and *Tradescantia*, but also to agricultural plants. Apparently the toxicity of solution 1A to tomato, alfalfa, and corn is in the order named.

While the box used for transferring the sterile seeds to sterile flask cultures is fairly efficient, the method is somewhat slow and tedious, and it is believed that fairly good results may be obtained without its use. It seems very desirable to extend our knowledge of the growth of seed plants in the absence of other organisms.

In the one case mentioned, the toxicity of the products of this rhizome to corn seemed to be independent of the presence of organisms, either at the time of dissolving the toxin from the rhizome, or at the time of its action on the growing plants.

The fact that the toxicity of solution 1A, even when undiluted, can be removed by precipitation with ammonium sulphate seems to suggest that the presence of colloidal matter may be a considerable factor in toxicity of that solution.

An alkaloid similar to nupharin is reported by WEHMER (**28**) in the rhizome of *N. alba*. He also reports fat and organic acids. These also represent possible factors in the toxicity. It seems possible that the toxicity may be due partly to products formed during decay and partly to products merely released by this decay.

The toxicity of the water extract of the ash is possibly accounted for on the basis of the presence of one or more basic substances. WEHMER states that the rhizome of *Nymphaea alba* contains

9.86 per cent of ash with the following composition: CaO, 8.2 per cent; Cl, 15.2 per cent; Na_2O, 48.47 per cent; K_2O, 9.86 per cent; P_2O_5, 14.36 per cent. The presence of sodium hydroxide or of sodium carbonate in the water solution of this ash seems probable.

There seemed to be 5 possibilities as to the cause of the toxicity of the products of the decay of this rhizome: (1) the presence of the organisms; (2) toxicity due to ionization; (3) the presence of toxic molecules; (4) high osmotic pressure of the solutions; and (5) low surface tension of the solutions. Of these, (1) seems to be practically eliminated by the work here reported. Apparently the presence of organisms, either at the time of the formation of the solution or at the time of their action on growing plants, is not a condition necessary for their toxic action. Work elsewhere reported (**20**) disposes of (4) and (5). This leaves ionization and toxic molecules as probable causes of the toxicity. The relative importance of these two is not fully determined by the work here reported. It is evident, however, that the entire toxicity cannot be ascribed to one substance. If we should suppose that the toxicity of all of the solutions obtained from this rhizome was due to only one substance, it would have to have the following properties: (1) soluble in water; (2) soluble in ether; (3) volatile at 40° C., 20 mm. pressure; (4) stable at 250° C., 15 lbs. pressure; (5) absorbed by animal charcoal; and (6) precipitation by ammonium sulphate.

It seems probable that there are at least 3 classes of substances here that are somewhat toxic: (1) colloids, (2) very volatile substances, and (3) certain bases.

Summary

1. The products of the decay of *Nymphaea* rhizomes are toxic to *Tradescantia* cuttings, and to tomato, alfalfa, and corn, even in very dilute solutions.

2. All of the solutions prepared except the one from the ash were acid, but the amount of acidity was not proportional to the degree of toxicity.

3. The toxicity of the very dilute solutions (but not of the strong solutions) was nearly destroyed by neutralization with sodium hydrate.

4. The toxicity of solutions resulting directly from the decay of the rhizome is destroyed by shaking them with animal charcoal.

5. The water extract of the fresh rhizome made under 15 lb. pressure in an autoclave is toxic to growing plants.

6. The rhizome and the products of its decay contain some ether-soluble toxic material.

7. Some of the toxic material in the products of the decay of these rhizomes is volatile at 40° C.

8. The toxicity of even concentrated solutions resulting directly from the decay of these rhizomes can be removed by precipitation with ammonium sulphate.

9. The water extract of the ash from these rhizomes is basic and toxic.

10. The products of the decay of potatoes, of turnips, and of the rhizomes of *Castalia odorata* and *Typha latifolia* are also toxic to *Tradescantia*, but slightly less so than those of *Nymphaea*.

UNIVERSITY OF WASHINGTON
SEATTLE, WASH.

LITERATURE CITED

1. ARCHICHOWSKY, V., Die Wirkung der Giftstaffe verschiedener Konzentrationen auf die Sammen. Biochem. Zeit. **50**:233–244. 1913.
2. BAUMAN, A., and GULLY, E., Über die freien Humussauren des Hochmoors. Mitt. K. Bayr. Moorkulturanst. **4**:31–56. 1910; see also Science N.S. **40**:492. 1914.
3. BOTTOMLY, W. B., The significance of certain food substances for plant growth. Ann. Botany **28**:531–540. 1914.
4. BROWN, A. J., The selective permeability of the covering of the seeds of *Hordeum vulgare*. Proc. Roy. Soc. **81B**:82–93. 1909.
5. CZAPEK, F., Über eine Methode zur direkten bestimmung der Oberblächenspannung der Plasmahaut, von Pflanzenzellen. Jena. 1911.
6. DACHNOWSKI, A., Bog toxins and their effect upon soils. BOT. GAZ. **47**: 389–405. 1909.
7. ———, Physiological properties of bog water. BOT. GAZ. **39**:348–355. 1905.
8. DANDENO, L. B., Mutual interaction of plant roots. Report Mich. Acad. Sci. **11**:24–25. 1909.
9. Exper. Sta. Record **15**:780. 1903–1904.

10. FITTING, H., Die Wassersorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitsch. Bot. **3**:209–275. 1911.
11. HARRISON, F. C., and BARLOW, B., The nodule organization of the Leguminosae. Bakt. Centralbl. **19**:264–272, 426–441. 1907.
11a. JENSEN, G. H., Toxic limits and stimulation effects of some salts and poisons on wheat. BOT. GAZ. **43**:11–44. *figs. 34.* 1907.
12. LIVINGSTON, B. E., Studies on the properties of an unproductive soil. Bull. 28, Bur. of Soils, U.S. Dep. Agric. 1905.
13. ———, Further studies on the properties of unproductive soils. Bull. 36, Bur. of Soils, U.S. Dep. Agric. 1907.
14. ———, Physical properties of bog water. BOT. GAZ. **37**:383–385. 1904.
15. LYON, T. L., and BIZZELL, J. A., Is there a mutual stimulation of plants through root influence? Jour. Amer. Soc. Agron. **5**:38–44. 1913.
16. MOILLARD, M., Sur la secretion par les racines de substances toxiques pour la plantes (note préliminaire). Bull. Soc. Bot. France. 1914.
17. NIKITINSKY, J., Beeinflussung der Entwickelung einiger Schimmelpilze durch ihre Stoffwechselprodukte. Jahrb. Wiss. Bot. **40**:1–67. 1904.
18. RAHN, O., Über den Einfluss der Stoffwechselprokte auf das Wachstum der Bakterien. Centralbl. Bakt. II. **16**:417–429, 609–617. 1906.
19. RIGG, G. B., The effect of some Puget Sound bog waters on the root hairs of *Tradescantia*. BOT. GAZ. **55**:314–326. 1913.
20. RIGG, G. B., TRUMBULL, H. L., and LINCOLN, MATTIE, Some physical properties of certain toxic solutions. To be published in the BOTANICAL GAZETTE.
21. SCHREINER, O., The organic constituents of soils. Science **36**:577–587. 1912.
22. SCHROEDER, H., Über die Einwirkung von Silbernitrat auf die Klimfähigkeit von Getreidkornern. Biol. Centralbl. **35**:8–24. 1915.
23. SHERFF, E. E., The vegetation of Skokie marsh, with special reference to subterranean organs and their interrelationships. BOT. GAZ. **53**:415–435. 1912.
24. SKENE, MACGREGOR, The acidity of sphagnum and its relation to chalk and mineral salts. Ann. Botany **29**:65–87. 1915.
25. SKINNER, J. J., Illustration of the effect of previous vegetation on a following crop. Plant World **16**:342–346. 1913.
26. SULLIVAN, M. X., The passage of nucleic acid from plant to medium. Science **39**:958. 1914.
27. WEHMER, C., Kleinere mykologische Mitteilungen. Centralbl. Bakt. II. **3**:102–108. 1897.
28. ———, Die Pflanzenstoffe. Jena. 1911.
29. WIELER, A., Pflanzenwachstum und Kalkmangel im Boden. 8vo. pp. vii+235. *figs. 43.* 1912.
30. WILSON, J. K., and HARDING, H. A., Method of keeping bacteria from growing plants. Science **33**:544–545. 1911.

NUCLEAR DIVISION OF SPIROGYRA

II. NUCLEAR DIVISION IN S. BELLIS

MABEL L. MERRIMAN

(WITH PLATES XVIII–XX)

In investigating the nuclear division of *Spirogyra* three modes of procedure were used. Study of whole mounts fixed and stained gives a better idea of disposition of chromatic material, whereas a study of sections gives a better idea of its structure. For estimating duration of phases in karyokinesis and relative activity of parts of nucleus and boundaries of nucleus and cytoplasm, it is necessary to study living nuclei.

Although many species have been worked upon, none has yet been found that presents favorable material for all these methods. If nuclei are large, as in *S. crassa*, and so adapted for dissection from whole mounts or for sectioning, then in a living cell close winding of the chromatophores prevents a view of the nucleus in karyokinesis. Hence the study of *S. crassa* (**8**) was confined to the study of sections and entire nuclei dissected from the threads. In the studies of *S. bellis* a correlation was attempted of nuclear division stage by stage as seen in living cells with similar stages fixed the same evening. The nucleus of this species is plainly visible when living, and when stained its density is not so great but that its structures may be seen readily without sectioning. The stains used were safranin and gentian violet; Haidenhain's hematoxylin with iron alum; and anilin blue with eosin. Since gentian violet stains the cell sheath as well as the chromatic material, the last two combinations of stains gave the best results.

Material in good condition was obtained from three different sources. It is of interest that in these materials from widely separated localities uniform variations existed, yet all can be included under one type, a type differing markedly from that of *S. crassa*, already published, and from that of *S. dubia* and *S. ternata* now in preparation. In regard to the nuclear division of *S. dubia*, the discovery of 5 chromosomes was made, while in *S.*

ternata 4 chromosomes were found. This would seem to be the lowest number yet found in plants. The following quotation from DIGBY'S (3) investigation on *Crepis virens* states: "*Crepis virens*, as has been shown by ROSENBERG, possesses only 6 somatic chromosomes, the lowest number hitherto recorded in plants." Six chromosomes are also recorded by WISSELINGH (12) in *S. setiformis*, and by KARSTEN (5) in *S. jugalis*. The number of chromosomes in *S. bellis* agrees with that found by the writer (8) in *S. crassa*, namely, 14. Since a single genus of plants, as shown in *Spirogyra*, exhibits such striking differences in the number of chromosomes, there is no foundation for the assumption that nuclei in plants of a similar genus will show similar morphological organization. Morphological variation in the nucleus obtains in different species, just as variations occur in the number and winding of chromatophores and in cell dimensions.

In the 3 varieties of *S. bellis* also minor variations were seen in karyokinesis. So far as known to the writer, such variations in nuclear division have not been worked out in detail and established for any particular plant. MITZGEVITSCH (10), working on *S. subaequa* and *S. jugalis*, found differences in the distribution and origin of chromatic material in the prophase; but to establish distinctions in nuclear division, varieties in species as well as many species for comparison should be worked upon.

The dimensions of the vegetative cells of these 3 varieties of *S. bellis* Hass., and conforming to *S. bellis* as described by COLLINS (2), are as follows:

Var. *A*.—Vegetative filaments 57 μ in diameter, 95 μ in length, 3 or 4 spirals in the filament. This was gathered from the margin of a brook in which was intermingled *S. longata*, *S. inflata*, *S. gracilis*, at Northfield, Massachusetts.

Var. *B*.—Vegetative filaments 64 μ in diameter, 220 μ in length, 5 spirals in the chromatophore, the latter markedly dentate; border of river in Needham, Massachusetts. This was not a pure culture of *Spirogyra*, but intermingled were various filamentous desmids and *Mougeotia*.

Var. *C*.—Vegetative filaments 60 μ in diameter, 220 μ in length, uniformly longer than *B* and much longer than *A;* some threads

with 4 spirals, others with 5; fertile cells not swollen, zygospore oval. It also resembles *S. majuscula* Wolle. This material grew in pure culture in a swamp in a low-lying pasture.

Preparations of the 3 varieties show essentially the same structures. *C* was noteworthy for great variation in number and size of nucleoli. The 3 varieties show striking uniformity in formation and appearance of chromatic disks in approximately the same numbers. It was found advisable to make sketches of nuclei not at equal intervals of time, but at those periods when changes in the form and density of the component parts were more manifest. As some of these changes took place with great rapidity, others only appearing as a slow evolution, many rough sketches were made, not only following one nucleus throughout its changes, but also in different nuclei, studying particular phases repeatedly where changes were most rapid, to confirm interpretations made upon one. This seemed necessary, as many of the appearances were at variance with published results of other investigators. The rapidity of the changes in the living material explains why we get such variation in the fixed material.

From the appearance of the first change in the nucleus until the close of the reconstruction of the daughter nuclei, about an hour elapses; in some cases, 80 minutes. As the phases merge into one another, it is difficult to give precise time, but the following schedule may be taken as a typical example: prophase 15 min., metaphase 5 min., early anaphase 15 min., late anaphase and telophase 30–45 min. The changes in the nuclei are most marked and rapid in the first 3 phases. In late anaphase and telophase, although changes in translucency and shape occur, they do not result in such great differences in position; hence frequent sketches with constant comparison of observations were necessary to be assured of these changes. Since the structural organization of the nucleus is but the result of fixation of colloidal materials seen in living cells, effort was made to find in the living cells the homologue of the stained structure.

The quiescent nucleus contains a central spherical body (fig. 1), or more often in variety *C* two or more such bodies. This body may appear bluish or slightly opalescent as compared with the

more translucent rim that extends into the suspensors. It may be subtended by a similar appearing but crescent-shaped mass (fig. 2) likewise surrounded by a translucent rim. In fixed material the central body of the quiescent nucleus shows still greater variations in appearance. One or more vacuolar-like appearances may be seen within, or these may be lacking, the space being filled with substances differentiated in the double staining. Sometimes the central body may be composed of many small granules uniformly deeply stained, although double staining in the cell has differentiated parts of the chromatophore.

That such a body, generally termed the nucleolus, is a nucleolus in every species of *Spirogyra* appears very doubtful to the writer. The position and relative amount of cytoplasm suspending the nucleus and forming a border to the same, the extent of nuclear plasm, nucleolus, and nucleolar vacuole exhibit great variations in the different species. Hence the suspicion arises that some of the discrepancies in results obtained by investigators such as TRÖNDLE (11) and ZACHARIAS (13), working on the microchemical reactions of the central body, may be due to the fact that in some species the central body is a nucleus, the considerable space about it being cytoplasm; while in other species only a narrow border of cytoplasm exists, the nucleus with the contained nucleolus comprising all. The limits of this paper will not permit the extended comparisons of different species that would serve to establish such a view. The term central body will be used in preference to that of nucleolus, the main object here being to present certain new details in the history of the chromatic figure.

Prophase living

Although the central body appears to become diffused in enveloping plasm, as preliminary to this process an infusion of cytoplasmic substance flowing in through the suspensors must have taken place to account for the enlargement of the mass. This inflowing substance mixes with the nuclear plasm and denser substance in the spherical body, causing the sudden overflow as it were of the boundaries of the sphere. This change takes place with great rapidity, as shown by comparison of figures, beginning

with one drawn at 8:50 P.M., where a nucleus with its barriers still unbroken can be seen, the larger granules vibrating above and below, with the same nucleus drawn 10 minutes later, where the previously spherical body has become completely diffused in the enveloping plasm. While this change takes place, the merged mass of central body and enveloping plasm is no longer spherical, but exhibits amoeboid movements (figs. 3, 4, 14), the various parts appearing to change constantly in density. In some cases there seemed to be no streaming of the larger granules, the latter appearing either stationary or oscillatory. The overflow of nuclear content is but the manifestation of the disturbance of the ratio of nuclear mass to cytoplasm advanced by HERTWIG (4) as the cause of karyokinesis. This enlargement of the nucleus is coincident with the increase on all sides in the size of the suspensors (fig. 4). This appearance suggests that the suspensors act as the main channels, enlarged now as inlets for the superabundant assimilatory products derived from the chain of pyrenoids with which they are connected.

Finally, those suspensors which attach the nucleus to the chromatophores in the long axis of the cell appear to enlarge more rapidly than the others, showing that the main currents are now diverted in this direction. Meanwhile the large vesicles, gathered above the nucleus and appearing to aggregate there in a plane through the short axis of the cell, show active Brownian movements.

During this constant amoeboid movement of the more or less spherical mass, the turbidity which before extended through the mass to the bordering translucent rim now appears to clear in certain regions (fig. 5). It is to be noted, however, that in this turbid mass there is no trace of organization. Regions where changes take place are indicated in the drawings, where turbidity is shown by the gray shading. Lessening of the turbidity is to be seen, not at the equator, but at the lighter areas at some distance and also in the regions near the bordering rim.

Meanwhile, this whirlpool-like activity shapes the spherical mass into that of an ellipsoid. The granular folds of protoplasm that were constituents of the suspensors, and that lay at the margin of the more homogeneous protoplasm enveloping the

central body, appear to be pushed back on either side by the interior expansion of the mass, finally to take up a position at the poles of the spindle (figs. 15, 16).

Prophase fixed

Since each preparation could exhibit only the particular phase of activity at the instant of fixation, and since this period averages 15 minutes, it would be difficult to find two series of precisely equivalent stages. The differences manifested upon fixation of the turbid spherical mass can best be explained by references to the figures. These all show with the enlargement of the nucleus the unequal increase of the suspensors as the spindle begins its evolution. In all of the cells the beginning of the formation of the cell plate is to be seen before the dissolution of the nuclear membrane. No attempt is made to present the figures in sequence, as there is no evidence as to whether one stage follows another or as to whether in other nuclei a different disposition of substance may not obtain at corresponding intervals of divisions.

The chromatic granules, as in fig. 28, may be scattered on the main suspensor as well as over the central mass of granules. All of the chromatic substance may be gathered in a sphere to one side of the enlarged nucleus. This substance is in the form of granules (fig. 27) or of filaments and granules (fig. 25). In fig. 26 chromatic granules are connected by a finer network and distributed all over the enlarged nucleus. In the center lighter granules may be seen, suggesting a decomposing nucleolus. Fig. 29 shows lighter stained granules equally distributed over the mass. Scattered among them are short filamentous bodies. Finer granular masses in the center indicate the remains of a nucleolus. In figs. 31 and 33 is seen a somewhat contracted spherical mass, evidently both nucleolus and nuclear plasm, and consisting of granules both lightly and deeply stained. These are connected with the nuclear membrane by delicately stained strands. This and fig. 25 might be considered as a stage in synapsis. In fig. 24 no finer granular material is seen within the nuclear membrane, but there are chromatic masses ranging from tetrahedral forms to that of vesicles. Fig. 35 shows similar chromatic masses imbedded in or

overlying a less deeply stained material. Other figures show minor variations from those previously mentioned. In comparison, all that these stages appear to have in common is the increasing tendency of the material, chromatic and non-chromatic, to be peripherally arranged on a sphere.

Metaphase living

As the enveloping protoplasm gradually becomes more tenuous, adding substance to the retreating folds, the ellipsoid mass changes and becomes cylindrical. This appears to be due to the accumulation of denser portions on the surface and the gradual penetration of more liquid material to the interior. This may be forced out at the poles as the denser materials assume the form of a disk (figs. 5, 15, 16). The cylinder as it elongates loses progressively its turbid appearance. As the turbidity diminishes, the equatorial part retains the gray tint, forming gradually a dark band (fig. 17), while light bands by degrees evolve, encompassing the cylinder one on either side of the equator (figs. 16, 18). These bands as they assume concrete form acquire a translucent appearance. They condense into two disks connected by fragments of cords of a similar translucent appearance. It is to be seen, therefore, that instead of a sharp splitting and consequent clear-cut separation of equatorial masses of the cylinder occurring, no actual narrow rift was perceptible, although many nuclei at this stage were closely scrutinized for the expected splitting. There is no evidence in living material of these two disks having arisen from the splitting of discrete chromatic bodies. It is as if a gradual accumulation and rearrangement of materials had taken place, until finally the dense materials, appearing as jelly-like disks, are moving to the poles of the spindles, while vestiges of cords drag behind (figs. 19, 20).

Metaphase fixed

Figs. 34 and 35 represent transitional stages where the nuclear membrane has dissolved. Here the mass of stained material is beginning to lose its spherical shape; and the suspensors change, some in the long axis of the cell increasing and appearing as lines of granules directed, not as before, away from the margin of the

nucleus, but from the darkly stained mass. Around each of the deeply stained bodies a faintly defined areola can be seen (fig. 36). In subsequent stages, instead of being disposed without order on the periphery of the mass, they seem rather to form an equatorial band around the cylinder (figs. 37, 38). Those which seem to be well defined, and hence possibly properly called chromosomes, average 14 in number. They may become looped and arranged in such manner that ends of the loops may present an appearance as if constituting a single row of granules (fig. 40). The other granular material composing the cylinder now shows a tendency to longitudinal striation (figs. 41, 42, 43); later it takes the form of pyramids (figs. 46, 47, 48). When the masses take the form of pyramids the apices of the pyramids point to the poles of the spindle. The edges of the pyramids may stain almost as black with chromatin stains as the other bodies (figs. 46, 47). Comparison of living materials with stages showing pyramidal arrangement of chromatic substance suggests that the pyramids, with their apices always pointing to the spindle poles, are but fixations of the streams emanating from the oppositely charged jelly-like opalescent disks. A spindle inclined by pressure shows, as the mass condenses, small thickenings of chromatin appearing at the edge of the substance (fig. 39); hence the thickenings are not confined to the denser filaments in the bands. Again, in many sections the pyramidal appearance of the chromatic material suggests in its orientation an incompletely formed spireme (fig. 47). At this stage the pyramidal masses discharge droplets of material from the edge of the equatorial band (figs. 47, 52).

A gradual amalgamation and condensation of the two substances next occurs. The looplike nature of the lighter stained material and the pyramidal appearance are brought out in figs. 46 and 47. Analyses of these figures show that as they amalgamate they form groups, each group consisting of 4 masses inclosing a vacuole. Such structure of groups is like that described for groups making up chromosomes in *Allium* (9). Comparison of fixed cytoplasm shows that granules in the cytoplasm often assume this form. Its frequent occurrence shows that we have not a splitting of materials; hence such appearances could play no important part in theories

as to the reduction of chromosomes. This is probably, as LILLIE (6) suggests in regard to tetrad groups of *Ascaris* described by BRAUER, a purely physical phenomenon, a grouping due to precipitation of oppositely charged colloidal masses. These groups in turn are connected with each other by strings of less dense substance. A side view of them gives the impression of short dark bodies subtended by loops of lighter substance. They may present the same pyramidal appearance as seen before the separation of the disks. Pressure on the cover glass on turning the disks to full polar view shows the disks to be of no appreciable depth, and to consist approximately of 4 rows of tetrads (figs. 56, 57). As the disks exhibit a tendency to be attracted to the poles they become cone-shaped, the apex pointing in the long axis of the cell.

A gradual pulling apart of the amalgamated material follows, until, as in figs. 53–57, two opposing disks are seen. The position of these disks, their shape, and consequent behavior in pulling apart, show them to be but the fixed and stained masses which constitute the jelly-like bands seen in living material evolving from the turbid mass. The turbid mass corresponds to the fixed spherical mass composed of irregularly disposed filaments or granules. These disks of material as seen in fixed specimens are not preceded by the splitting of chromosomes in prophase, as stated by MITZGEVITSCH (10), BERGHS (1), and others. It is the amalgamated masses just as in *S. crassa* (8), the amalgamated spireme that appears to be pulled apart, forming two opposing networks in which no distinction of material now is apparent, unless it be in the linin-like connections of the groups.

With the formation of these networks, the space between the two becomes very clear. All the granules making up the spindles are absent, the intervening space between the networks being crossed by a few strands (figs. 51, 52) which in later stages appear to become attenuated and then disappear. These strands are identical with the translucent cords which in living material connect the separating jelly-like disks. Studies of living material raise the question whether the matter in the disks may not move more often to the poles in strands or streams not cohering in a disk, but reassembling in the form of disks when the poles are reached.

The network as it passes to the poles is seen to be not a uniform disk, but to become concave (fig. 55), the concavity pointing to the poles, indicating the direction of the attractive force. In some cases the concavity appears as a cone of granular material on a base of deeply stained tetrahedral masses (fig. 58). Arrived at the poles the network now converges to a spherical shape (figs. 59, 60), the chromatic material tending to become peripheral in position (fig. 62). Irregular masses of it later may be discharged. Fig. 67 illustrates the intimate connection of chromatic substance with pyrenoids. Figs. 62–68 show that as many variations mark the beginning of telophase as were seen in prophase. The chromatic masses become reduced in size, while the nucleolus as a reserve body appears in their midst.

Since the increase in size of the nucleolus is correlative with the reduction in chromatic masses, it is probable that it is not to be considered a karyosome, but derived indirectly from them.

Anaphase living

Returning to the study of living material, we find that this can be correlated step by step with that seen in fixed material. The movement of denser portions of disks away from each other does not always occur at once, as strands of the translucent substance connect the separating masses and also strands soon form connecting the granular polar masses (figs. 7, 17–20). These dense strands show no evidence of being composed of homogeneous bodies arranged as strings of beads. This accords with the observations on living material made by LUNDEGARDH (7).

While the translucent disks that we may term *a* and *b* are evolving and gradually retreating from one another, a similar (in appearance) jelly-like substance *c* and *d* appears at either end of the cylinder as it assumes the typical spindle form (fig. 7). Disks are represented by light areas. These evidently are not directly derived from the disks, as they are of considerable size before the approach of the latter; also, the disks do not appear to diminish as the strands bordering the cylinder increase. The ends *c* and *d*, partly under the chromatophore, show the same optical density, and are translucent and opalescent, while all else is grayish. The

substances *c* and *d* are evolved about 5 minutes later than the first appearance of *a* and *b*. Upon examining many kinds of fixed material, stages were found, as shown in figs. 38, 53, and 55, where polar chromatic disks similarly appear in metaphase and anaphase. In some cases only chromatic granules, in place of disks, are present, reminding one of centrosomes (fig. 50).

These substances appearing at the ends of the spindle may have been originally either at the border of the turbid mass seen in prophase (fig. 5), or else may be the accumulations discharged from the disk in metaphase as separate droplets (see fig. 62, from fixed material). Whether of cytoplasmic or nuclear origin, they appear to be of the same consistency as the disks having similar indices of refraction. The ends of the spindle are now lost to view in aggregations of granular matter that appear identical with the earlier suspensors. As the disks *a* and *b* approach the poles, a border of similar material appears, connecting them with disks *c* and *d* (fig. 8). This results in two irregularly shaped figures inclined to the quadrilateral, the jelly-like substance on the rim, the interior grayish.

This blending takes place before the two gels in their retreat have reached the polar granular masses. The substance in *c* then, probably contributed by the cytoplasm, rejuvenates the chromatic substance, and with this blending the disks likewise lose strands of their material to the cytoplasm. The changes in form and translucence of all substances in this stage take place with great rapidity. This accounts for the great variability and amorphous appearance of the separating disks as seen in prepared slides. The many kaleidoscopic shiftings of these masses, as illustrated in figs. 8–11 and 20–22, result in the appearance of a nucleolus within the grayish interior of each quadrilateral, while the gel forms the rim or daughter nuclear plasm. As movements subside, a reversal of optical refraction ensues, the central body appearing to increase in density, while the nuclear plasm becomes optically more like the hyaline cytoplasm (figs. 12, 13).

The daughter nucleus has manifestly received accessions from the cytoplasm at two periods in the karyokinesis: (1) when the sphere enlarges and becomes turbid; and (2) when disk *c*, apparently

from the cytoplasmic material, blends with *a* from the equatorial disk. Since disk *c* with *a* plays an important part in the reconstruction of the nucleus, and only disk *a* seems to coincide with the amalgamated chromatic material seen on the slides, it would appear that *Spirogyra* lends support to the views of those who believe that the chromosomes are not the sole containers of hereditary substances. It cannot be said that the material of the mother nucleus is equally divided between the two daughter nuclei, for all of the material in the two nuclei is not from the mother nucleus; while again, some of the colloidal material of the mother nucleus passes into the cytoplasm before the two daughter nuclei are formed. These observations also suggest that karyokinesis is no longer to be considered merely as a process of division, but as a process made up of alternating phases of addition, combination, and withdrawal of protoplasmic substances from nuclear centers.

Summary

Instead of a spireme, as in *S. crassa*, a disk arises from material condensing within the mass of nuclear plasm and central body. This disk is discernible in both living and stained material.

No trace of organization is to be seen in the living disk, but fixed material shows it to arise from aggregations of variable appearance and staining qualities. These aggregations are not the chromosomes. The more deeply stained of these bodies arise from the nuclear plasm, the less deeply stained appear to come from the decomposing central body.

This sphere of aggregated material gradually changes in shape, becoming a cylinder. The more deeply stained masses become arranged upon it as an equatorial band. This band is homologous with the disk seen in living material. As the disk evolves, chromatic bodies, averaging 14 for this species, are to be seen on the band, while other irregular masses of chromatic material project as loops or pyramidal masses from its edge. These loops or masses represent material from nuclear plasm and central body that has partially amalgamated.

No rift appears in the living disk to indicate a sharp splitting of components, but instead the changes in appearance indicate a

thinning in the center, while parts reassemble at either pole. The chromatic bodies in the fixed disks appear as viscous masses that, as they amalgamate, elongate, while other disconnected chromatic masses are discharged into the cytoplasm as the disk separates into the halves passing to the poles.

The living disks may be seen sometimes to pass *en masse* to the poles, but more usually they divide their substance into a few continuous strands, to reassemble as disks at the poles of the anaphase. These strands cannot be identified as moving chromosomes, since no units can be discerned in them. As the disks approach the poles, they appear to blend with similar disks apparently evolved from cytoplasm.

Each daughter disk thus arising upon fixation consists of a series of about 4 rows of tetrahedral masses. In living material the same appears as a translucent rim surrounding a less dense interior. The translucent rim becomes the nuclear plasm, while the central body takes shape within the less dense interior.

Spirogyra, as exemplified in *S. bellis* and *S. crassa*, may be characterized as having chromatic substance of a polymorphous nature; in the one a disk, in the other a spireme. The nucleolus does not fragment directly into chromosomes, as upheld by so many investigators, but only contributes the less dense substance seen at metaphase, which eventually may be discharged or become partially amalgamated with the chromatin. Hence *Spirogyra*, as regards the constitution and behavior of its nucleolus, need not be placed in a different category from the remainder of the green algae or from that of higher plants.

HUNTER COLLEGE
NEW YORK CITY

LITERATURE CITED

1. BERGHS, J., Le noyau et la cinèse chez le *Spirogyra*. La Cellule **23**:53–86. 1906.
2. COLLINS, F. S., The green algae of North America. Tufts College Studies **2**:no. 3. 1910.
3. DIGBY, L., A critical study of the cytology of *Crepis virens*. Archiv Zellforsch. **12**:——. *pls. 8–10.*

4. HERTWIG, R., Über Korrelation von Zell und Kerngrösse und ihre Bedeutung. Biol. Centralbl. **23**: 49–62, 108–119. 1903.
5. KARSTEN, G., Die Entwickelung der Zygoten von *Spirogyra jugalis*. Flora **99**: 1–11. *pl. 1*. 1908.
6. LILLIE, R. S., The physiology of cell division. Amer. Jour. Physiol. **15**: 46–84. 1905.
7. LUNDEGARDH, H., Die Kerntheilung bei höheren Organism nach Untersuchungen an lebenden Material. Jahrb. Wiss. Bot. **21**: 236–281. 1902.
8. MERRIMAN, M. L., Nuclear division in *Spirogyra crassa*. BOT. GAZ. **56**: 319–330. *pls. 11, 12*. 1913.
9. ———, Vegetative cell division in *Allium*. BOT. GAZ. **37**: 178–207. *pls. 11–13*. 1904.
10. MITZGEVITSCH, L., Über die Kerntheilung bei *Spirogyra*. Flora **75**: 81–124. 1898.
11. TRÖNDLE, A., Der Nukleolus von *Spirogyra* und die Chromosomen von höheren Pflanzen. Zeitschr. Bot. **4**: 721–747, *figs. 11*. 1912.
12. VAN WISSELINGH, C., Über Kerntheilung bei *Spirogyra*. Flora **86**: 353–377. 1900.
13. ZACHARIAS, E., Die chemische Beschaffenheit von Protoplasma und Zellkern. Prog. Rei. Bot. pp. 67–257. 1910.

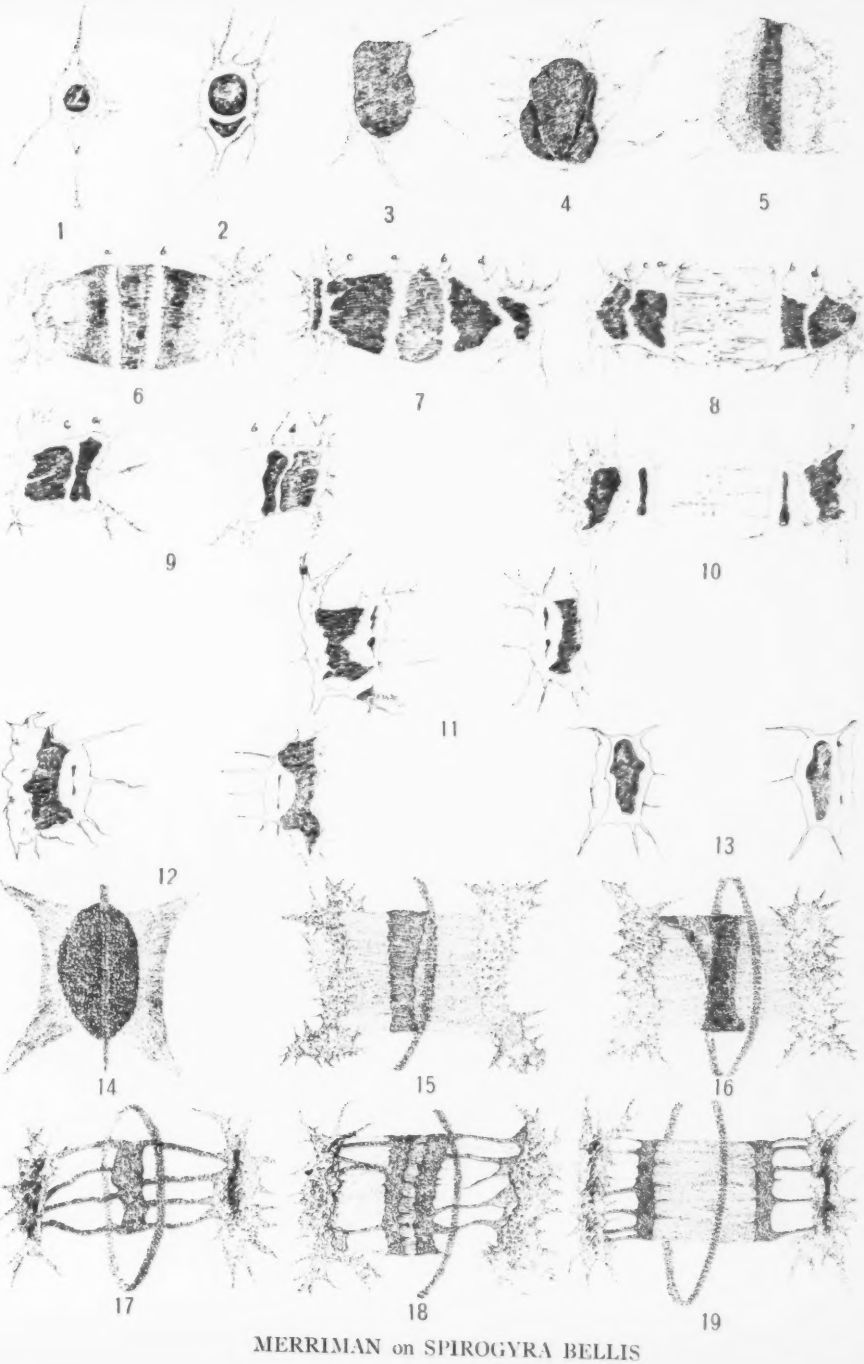
EXPLANATION OF PLATES XVIII–XX

All figures, except those of living nuclei, were drawn with the Abbé camera lucida, Leitz objectives, and Zeiss compensating oculars; drawings reduced one-half.

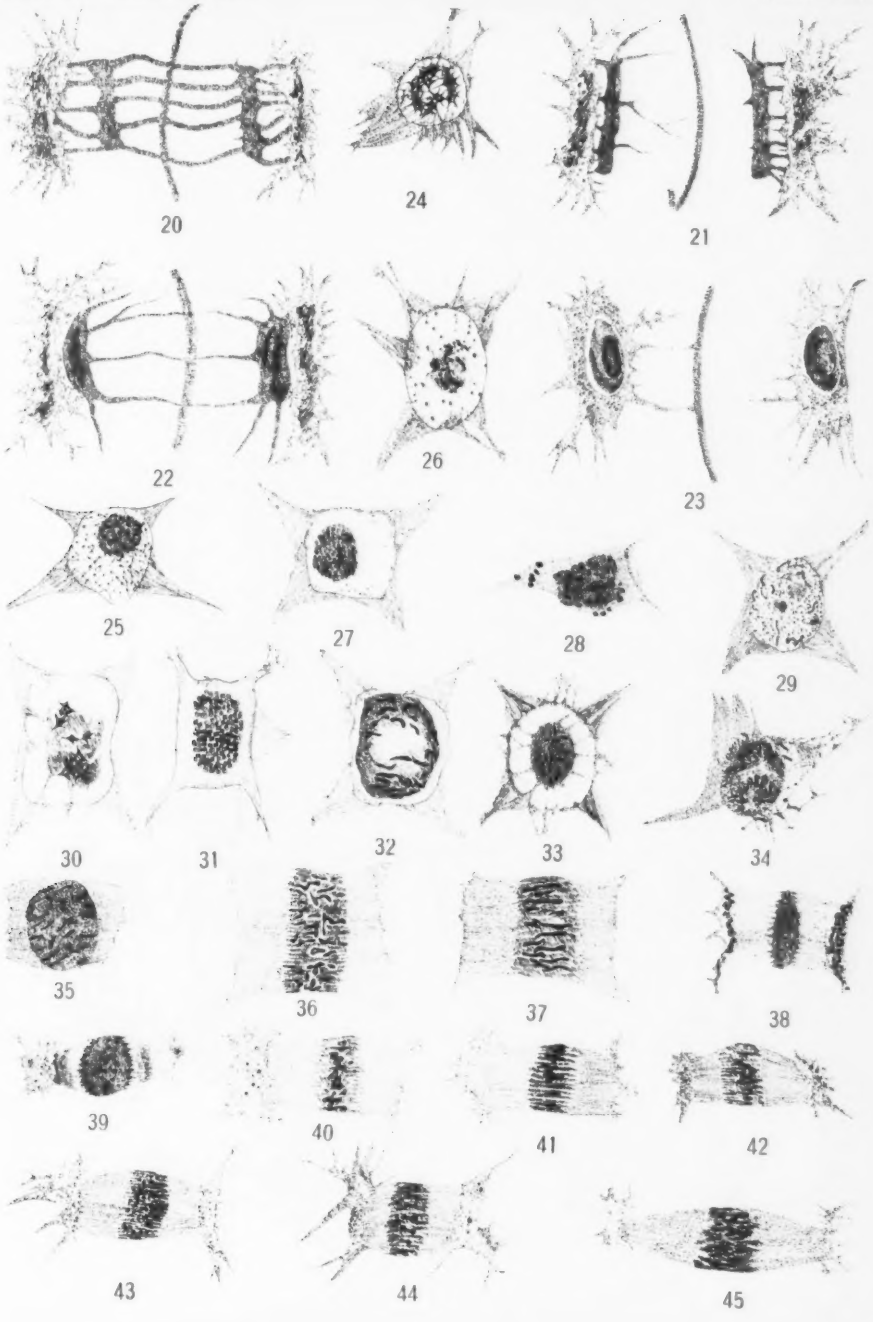
FIGS. 1–13.—Living nuclei of var. *C*: figs. 1 and 2, quiescent nuclei; fig. 3, early prophase at 9:07 P.M.; fig. 4, same at 9:15 P.M.; fig. 5, at 9:20 P.M.; fig. 6, at 9:25 P.M.; fig. 7, at 9:32 P.M.; fig. 8, at 9:36 P.M.; fig. 9, at 9:40 P.M.; fig. 10, at 9:44 P.M.; fig. 11, at 9:48 P.M.; fig. 12, at 9:52 P.M.; fig. 13, at 9:55 P.M.

FIGS. 14–23.—Living nuclei of var. *A*: fig. 14, at 7:45 P.M.; fig. 15, at 7:50 P.M.; fig. 16, at 7:55 P.M.; fig. 17, at 8:00 P.M.; fig. 18, at 8:07 P.M.; fig. 19, at 8:10 P.M.; fig. 20, at 8:12 P.M.; fig. 21, at 8:25 P.M.; fig. 22, at 8:28 P.M.; fig. 23, at 8:33 P.M.

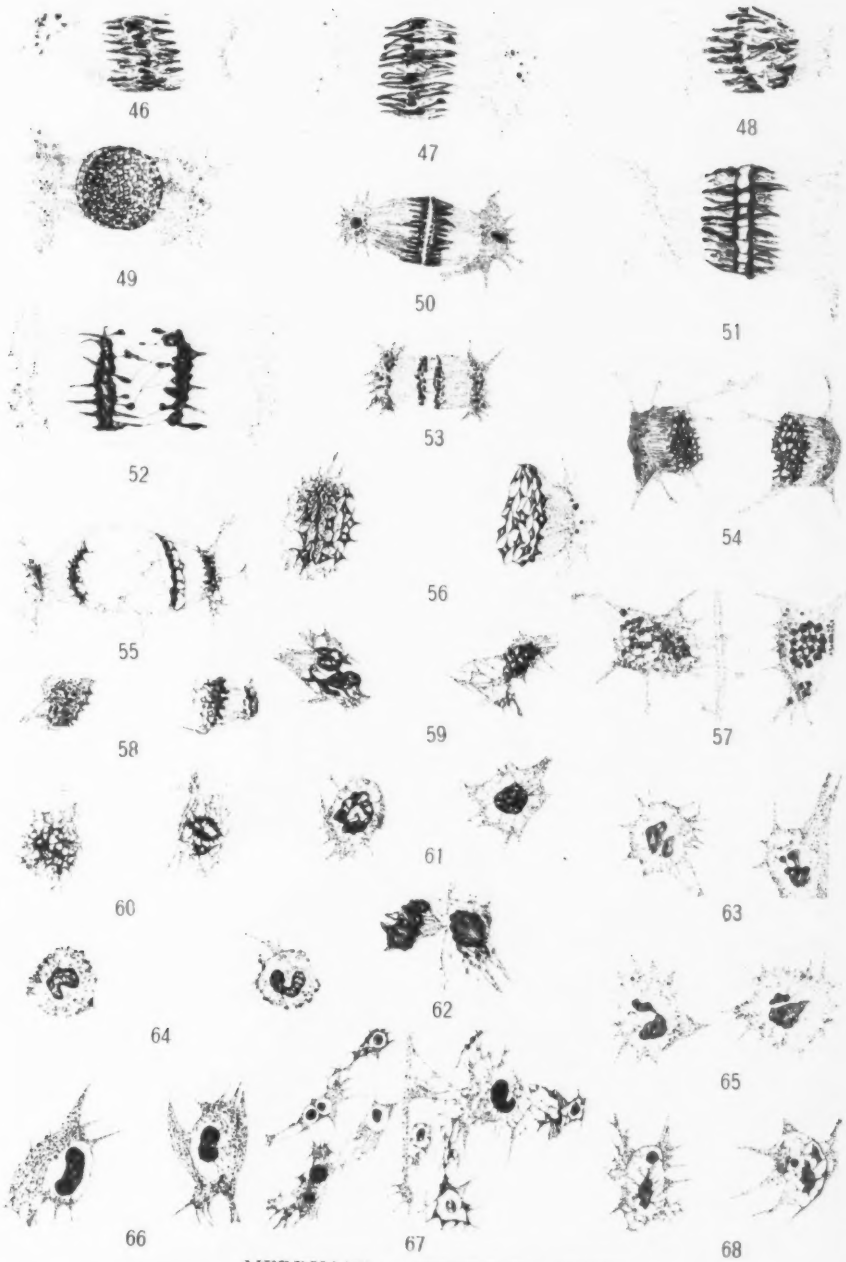
FIGS. 24–68.—From preparations fixed in chromo-acetic; figs. 36, 46–48, 51, 52, and 56×3000; all others ×1800.



MERRIMAN on SPIROGYRA BELLIS



MERRIMAN on SPIROGYRA BELLIS



MERRIMAN on SPIROGYRA BELLIS

THE EMBRYO SAC OF RICHARDIA AFRICANA KTH.

MARGARET R. MICHELL

(WITH PLATES XXI–XXIII)

Up to the end of last century very little was known of the embryo sacs of the different genera of the Araceae. Since then, however, several American botanists have contributed to our knowedge of the subject, with the result that now we do know something of the development of the embryo sacs. Yet in spite of this work, our knowledge of the family is by no means satisfactory. Perhaps this may be due to the fact that much of the material worked upon has been collected in greenhouses, where conditions are far from normal. The difference in habit between the plants of *Richardia africana* growing in their home in South Africa and those found in greenhouses in England is most striking, and it is possible that conditions which can affect the outward appearance of the plants to such a large extent may be effective in producing abnormalities in the embryo sac.

While this investigation was in progress, GOW (**7**) published a short account of the embryo sac of *Richardia*. His results differ from mine in so many respects, however, that it seems advisable to publish my results, particularly as my material was obtained from plants in their native habitat.

Richardia africana is a native of Cape Colony and St. Helena (**5**), and flowers freely in damp open places in the Cape Peninsula, where the material for this investigation was collected. The plants are in full flower in August, although young inflorescences may be obtained as early as April.

The chief fixatives used were an alcoholic solution of corrosive sublimate and picric acid, made according to JEFFREY'S formula: Carnoy's fluid and a mixture of three parts of absolute alcohol to one part of glacial acetic acid. A certain amount of shrinking took place in all cases, though in the very young ovaries fixed in the absolute acetic mixture this was practically negligible. Chrom-acetic solutions gave very poor results. The chief stain used

throughout was Haidenhain's iron-haematoxylin, as this gave excellent differentiation in all stages. Good results in the post-fertilization stages were obtained with carthamine (gossypimine) and picric-aniline blue. Many other stains were tried, but none of them was as good as the two already mentioned.

Ovule and embryo sac

The ovary usually consists of 3 carpels and is trilocular, each loculus containing about 4 not very definitely anatropous ovules arranged in an axile manner. These ovules have the massive basal part of the outer integument so characteristic of the Araceae. The inner integument closes about the time that the megaspore mother cell shows signs of the first division leading to the formation of the embryo sac. The outer integument remains open until endosperm development is well under way.

The two integuments show numerous changes throughout their course of development. The inner one does not attain any great thickness, never being more than 2 or 3 cells thick. Before fertilization, the cells of the innermost layer of this integument, especially those cells in the region round the lower part of the embryo sac, grow rapidly in a plane at right angles to the axis of the ovule (fig. 11*a*). After fertilization, cells of this layer again grow rapidly and gradually lose their flattened form, although they still remain conspicuous by reason of their large size. The chief feature of the outer integument is its great thickness in the chalazal region. It is composed of small uniform cells, with here and there a large cell containing raphides. These large cells are in every way similar to those found in the ovary wall. As the seed matures, intercellular spaces appear in the integument, and the outer surface becomes lobed, particularly at the micropylar end.

The nucellus, as in most other Araceae, is not very permanent tissue, and by the time the egg is ready for fertilization all that remains of it is a cap above the embryo sac and a few cells at the base.

The archesporial cell is differentiated early, before the origin of the second integument. It is easily distinguishable by its denser protoplasm and somewhat larger nucleus. Soon after the appear-

ance of the rudiment of the outer integument, the nucleus of this cell goes into synapsis. Fig. 1 shows the spireme loosening itself from the synaptic knot. The synaptic knot in *Richardia* is a very tight one, and in many ovules in this stage no trace of the spireme could be detected emerging from it. Fig. 2 shows a further stage in the heterotypic division, 12 bivalent chromosomes being arranged on the equatorial plate. The homotypic division follows, thus giving rise to a row of 4 megaspores. Fig. 3 shows stages in the anaphase of this division. In the nuclei represented in this figure it is interesting to note that in the upper nucleus the two halves of one chromosome have not separated, but have remained attached by one end. As a rule sluggish divisions of this type were not observed.

Of the 4 megaspores, only the lowest gives rise to the embryo sac. Fig. 4 shows an ovule in which the uppermost megaspore is beginning to degenerate. In certain cases in which the uninucleate embryo sac is a little larger than the one in this figure, only the remains of 2 megaspores could be detected. It is possible that in these cases only 3 megaspores were formed, although none of my preparations of stages before degeneration shows fewer than 4 megaspores.

Gow (**7**) states that the spore mother cell develops directly into the embryo sac and that a row of megaspores is not formed. In my material I found that a row of megaspores is invariably formed, and in no case is there anything to lead one to suppose that the embryo sac has originated directly from the megaspore mother cell.

The development of the embryo sac is perfectly normal. In the majority of cases there is a marked polarity. Fig. 5 shows the first division in the embryo sac. In the section represented, only 2 of the degenerating megaspores are to be seen, but a third is present in the next section. Fig. 6 shows the binucleate stage of the embryo sac, and fig. 7 the 4-nucleate stage. Fig. 8 represents the division of these 4 nuclei. It is unfortunate that this was the only case obtained in which the 4 nuclei were dividing, as the polarity so generally met with is absent, and it is not known whether this is the usual state of affairs or not. In subsequent stages shown in my preparations the embryo sac is markedly bipolar.

Fig. 9 shows the upper polar nucleus beginning to move toward the chalazal end of the embryo sac. The next stage is shown in fig. 10, in which the antipodals are showing signs of disintegration. The synergids have not assumed their mature form, but nevertheless may be distinguished from the egg by their position. Early degeneration of the antipodals is usually marked in *Richardia*, although in a few cases they persist, although somewhat degenerate in appearance, up to the time of endosperm formation. In nearly all my preparations showing the mature embryo sac, all that remains of the antipodals is a dark mass of degenerating cells which are hardly distinguishable from the nucellar cells, which at this period often show signs of breaking down. It was not until younger stages were procured that it could be clearly demonstrated that the embryo sac had 8 nuclei, and not 5, as CAMPBELL (**4**) has described for certain species of *Aglaonema*. However, the stages figured in figs. 8 and 10 prove conclusively that at one period the embryo sac has 8 nuclei.

The stage most commonly met with, and one which evidently persists for some time, is the 5-nucleate one, shown in fig. 11(*a*, *b*, *c*). Here the synergids are quite distinct from the egg, as the cytoplasm of the egg is very much finer. The 2 polar nuclei are imbedded in dense granular protoplasm at the base of the embryo sac, and these nuclei are by far the most conspicuous nuclei in the embryo sac, owing to their larger size and deeper staining capacity. The degenerate antipodals are to be seen at the base of the embryo sac. Figs. 11*a* and 11*b* show the halves of 2 of the 3 antipodals, while fig. 11*c* shows the third.

The 2 polar nuclei fuse some time before fertilization. Fig. 12 shows this fusion taking place in an embryo sac where the 2 nuclei are somewhat smaller than is usually the case.

The position of the polar nuclei at the base of the embryo sac is a constant feature of *Richardia*, and in over 300 slides showing the embryo sac in this stage, none shows the polar nuclei in any position save that at the base of the embryo sac. Gow (**7**) in fig. 39 of his paper depicts a mature embryo sac which is quite unlike anything that I have come across in my preparations. At the time of maturity the embryo sac has the appearance shown in

fig. 11*a*. As fertilization has been demonstrated at this stage, it is safe to conclude that the embryo sac is mature. It is possible that GOW's figure was taken from a younger ovule.

Fertilization

The process of fertilization is rather difficult to demonstrate in *Richardia*. Quite possibly it does not occur in the majority of ovules, as even in its native habitat *Richardia* does not set seed freely. It appears that this failure to set seed is seldom due to a lack of organization of the embryo sac, or to sterility of the pollen grains, but probably may be accounted for by the absence from the plants of an efficient pollinating agent. In this connection it is significant to note that it is rare to find one pollinated ovary alone on an inflorescence. If pollination has occurred, usually most of the ovaries have been pollinated.

CHURCH (**5**) has reported that small flies and green aphids visit the inflorescence when in cultivation in England, but adds that they do not seem to be of much service in pollination. The same thing, if earwigs be added to the list, occurs in the Cape Peninsula, where the remains of these insects are to be found at the base of inflorescences which obviously have not been pollinated.

A large number of ovaries were collected from inflorescences of widely differing ages. The embryo sac is mature by the time the spathe opens, and persists in this stage for an indefinite length of time. It has even been found in an ovary taken from an inflorescence whose spathe was withering.

Even if fertilization does not occur, the ovaries enlarge. It has not been possible, through lack of opportunity of proving it experimentally, to demonstrate whether the egg is capable of being fertilized the whole time the spathe is open, or whether the fertilization period is restricted to a longer or shorter time dating from the opening of the spathe.

Only one case of undoubted fertilization has been observed. This is shown in fig. 13, in which the pollen tube is seen passing through a synergid and the male nucleus is fusing with the egg nucleus. "Double fertilization" has not been observed. In the lower part of the ovule in which fertilization is being effected, the

primary endosperm nucleus has already divided, and thus there is no means of detecting whether a male nucleus took part in its formation or not. This early stage in endosperm formation is shown in fig. 4, where the antipodals are still distinct.

Sterile ovules

Gow (**7**) has reported that in *Richardia* as observed in the Cedar Rapids greenhouses, a large number of ovules are congenitally sterile, producing no embryo sac. In my South African material this does not seem to be the case. In a few ovules no functional embryo sacs were found, but judging from the fact that a group of degenerate cells could be distinguished in the nucellus in each case, it seems probable that an embryo sac had been formed and had collapsed.

Development of the embryo

Owing to the fact that fertilization had occurred in very few cases, ovules showing young embryos were only occasionally found. Endosperm development precedes that of the embryo, and by the time the small spherical embryo consists of 32 cells, the embryo sac is filled with endosperm.

In one case a 2-celled structure, which had every appearance of being an embryo, had developed at the chalazal end of the embryo sac (fig. 15*a*). Its protoplasm is fine and dense, making a marked contrast with the coarsely granular protoplasm around the endosperm nuclei which are found in the next section (fig. 15*b*). The position of the antipodals is indicated by the degenerating cells shown in figs. 15*a* and 15*b*. This was the only case in which an embryo was found in any but the normal position, and its origin is not known. In this embryo sac an egg was organized (fig. 15*c*), although it has evidently not been fertilized.

In all the other ovules the proembryos seen had at least 32 cells and were spherical in shape. Fig. 16 shows a somewhat oblique section passing through an embryo with over 32 cells. The embryo conforms to the type described by both CAMPBELL and GOW for the Araceae.

Endosperm formation

Endosperm formation proceeds from the base upward. Exactly when cell walls first appear has not been ascertained, but from a comparison of different stages in endosperm development it seems probable that they appear soon after the first 2 or 3 nuclear divisions. Fig. 14, which represents the chalazal end of the embryo sac in which fertilization has been demonstrated, shows the 2 nuclei resulting from the division of the primary endosperm nucleus. Fig. 15*b* shows the division of the lower of these nuclei. Fig. 15 shows the antipodals which have by this time lost all semblance of active cells. Perhaps it is hardly fair to quote this as an instance of the complete disorganization of the antipodals after endosperm formation, as in this embryo sac an abnormal embryo has been formed, but the same thing is seen in fig. 11 (*a*, *b*, *c*) and in fig. 17, which represents a slightly later stage in the development of another embryo sac, and in every preparation of embryo sacs made at this stage. It is clear that the antipodals disorganize rapidly, and in the embryo sacs shown in figs. 18 and 19 they are no longer recognizable.

The basal endosperm cells usually have a striking appearance. Three to five of these cells become much larger than any other endosperm cells, and may be seen with the naked eye (figs. 18 and 19). The protoplasm and nuclei of these cells also undergo some change. The protoplasm becomes coarsely granular and has a honeycombed appearance. All nuclear outline is lost, while the nucleolus hypertrophies, shows great vacuolization, and finally fragments, spreading its substance over practically the whole cell. The nuclei of the neighboring endosperm cells often imitate those of the giant cells in behavior. One thing is certain, and that is that these cells are true endosperm cells and have nothing to do with the antipodals.

The function of these cells is not clear. It is interesting to compare them with the antipodal cells described in *Tricyrtis hirta* by IKEDA (**10**). IKEDA believes that the cytological features exhibited by the antipodals in *Tricyrtis* bear a relation to their nutritive activity. This seems to be true for these endosperm cells of *Richardia*. Everything points to the conclusion that these

cells have a nutritive function. CAMPBELL has suggested that the massive basal part of the outer integument in the Araceae may be physiologically considered as perisperm. As both the inner walls of the outer integument and the embryo sac wall, except at its base, are cutinized, it is obviously impossible for food to pass into the embryo sac except through the enlarged endosperm cells. By the time the embryo is mature these cells have disappeared and much of the food material in the basal part of the integument has been absorbed.

It is a well known fact that cells which are active in nutrition possess nuclei which differ from nuclei in the ordinary resting condition in their larger size, their chromatin aggregations, and appearance of lack of organization. HUIE (**8, 9**) and ROSENBERG (**12**) have described this phenomenon in the gland cells in the tentacles of *Drosera*, while MAGNUS (**11**) has shown that the same thing occurs in the digestive cells of certain orchids having endotrophic mycorhiza. These facts seem to indicate that a nutritive function is to be ascribed to these basal endosperm cells.

Discussion

Great variety in development of the embryo sac, even within a single species, has been reported for the Araceae, and it was with a view to finding out in what respects *Richardia africana* resembled the other members of the family that this investigation was undertaken. *Richardia africana*, or *Lantedeschia aethiopica* as ENGLER (**6**) calls it, belongs to the Philodendroideae, to which group also belong *Homalonema*, *Philodendron*, *Aglaonema*, and *Dieffenbachia*.

According to CAMPBELL and GOW, all these genera, with the exception of certain species of *Algaonema*, show a normal 8-nucleate embryo sac. In a series of papers on the subject, CAMPBELL (**1, 2, 4**) has dealt with 4 species of *Aglaonema*. He finds that the embryo sac of *A. commutatum* may contain 4–12 nuclei, and shows little uniformity in arrangement. This species has a group of cells at the base of the endosperm bearing some resemblance to that in *Richardia*. In 1900 CAMPBELL suggested that these might originate by division of the antipodals, but later in 1903 inclined to the view that they were of endospermic origin. GOW (**7**), however, in

1913, without reference to the later paper, confirmed CAMPBELL'S view of 1900.

In 1912 CAMPBELL (4) published the results of his researches on *A. simplex* and *A. modestum*. He believes that there are only 5 nuclei in the embryo sac in these species, this stage arising from the further division of one of the micropylar nuclei in the 4-nucleate stage. The obstacle in the way of accepting CAMPBELL'S view is that he has never been able to demonstrate the supposed nuclear division. My investigations on the embryo sac of *Richardia*, which about the time of maturity has every appearance of being 5-nucleate, have led me to doubt the division of one only of the 4 nuclei.

In many cases the antipodals degenerate almost as soon as formed, and are indistinguishable from the nucellar cells, which also show signs of degeneration. However, it has been possible to show that in *Richardia*, at least, the embryo sac is at one stage 8-nucleate; and in the absence of definite proof to the contrary one cannot but feel that the same may be true for *Aglaonema*. In the fourth species *A. pictum*, CAMPBELL records the presence of cells which look like antipodals, although in the light of his work on *A. modestum* and *A. simplex*, he is inclined to believe that they are of nucellar origin.

In a comparison of *Richardia* with the other genera of the Araceae, mention must be made of *Spathicarpa*, the development of which has been studied by CAMPBELL (2). At the time when the embryo sac is filled with endosperm, it bears a striking resemblance to that of *Richardia*, but in *Spathicarpa* CAMPBELL derives the large cells at the base of the endosperm from the antipodals, which up to the time of fertilization are inconspicuous. In *Richardia* this is not the case, the antipodals being evanescent in character.

Summary

1. The ovary of *Richardia africana* is usually trilocular and has axile placentation. Four ovules are borne in each loculus.

2. The ovule is not very decidedly anatropous and has two integuments.

3. By the time the embryo sac is mature only a few cells at the apex and base of the nucellus remain.

4. The primary sporogenous cell gives rise directly to a row of 4 megaspores. The embryo sac is derived from the lowest of these.

5. An 8-nucleate embryo sac develops in the normal way.

6. The antipodals usually degenerate early, and when the embryo sac is mature often cannot be distinguished from the nucellus, which also undergoes a certain amount of degeneration.

7. The embryo sac persists for a long time in the stage when only 5 nuclei are distinguishable. The egg apparatus is normal and the 2 large polar nuclei lie in a mass of granular protoplasm at the base of the embryo sac.

8. The proembryo is spherical, with a minute suspensor.

9. In one ovule a 2-celled structure looking like a young embryo was found at the chalazal end of the embryo sac.

10. The endosperm develops from the base upward, and is probably accompanied by wall formation.

11. A few cells at the base of the endosperm are much larger than the rest. They possess hypertrophied nuclei and granular protoplasm. Their function is probably that of passing up food material to the young endosperm and embryo.

In conclusion I am glad to take this opportunity of thanking Dr. PEARSON for suggesting the work and for handing over to me his material and some preparations he had made. I am also indebted to Professor SEWARD for kind permission to carry on this investigation at the Cambridge Botany School, and to Mr. GREGORY for helpful criticism of this paper. I also wish to thank Miss E. L. STEPHENS, who verified certain points for me in connection with the pollination.

THE BOTANY SCHOOL
CAMBRIDGE, ENGLAND

LITERATURE CITED

1. CAMPBELL, D. H., Studies on the Araceae. Ann. Botany **14**: 1–25. 1900.

2. ———, Studies on the Araceae. II. Ann. Botany **17**: 665–687. *pls. 30–32*. 1903.

3. ———, Studies on the Araceae. III. Ann. Botany **19:** 329–349. *pls. 14–17.* 1905.

4. ———, The embryo sac of *Aglaonema*. Scot. Bot. Rev. 1912.

5. CHURCH, A. H., Types of floral mechanism. Part I. Oxford: Clarendon Press. 1908.

6. ENGLER, A., und PRANTL, K., Die Natürlichen Pflanzenfamilien **2**[3]: 1889.

7. GOW, J. E., Morphology of aroids. BOT. GAZ. **56:** 127–142. *figs. 47.* 1913.

8. HUIE, L., Changes in the cell organs of *Drosera rotundifolia* produced by feeding with egg albumen. Quart. Jour. Micr. Soc. **39:** 387–425. 1897.

9. ———, Further study of cytological changes produced in *Drosera*. Part II. Quart. Jour. Micr. Sci. **42:** 203–222. 1899.

10. IKEDA, T., Studies in the physiological functions of antipodals and the phenomena of fertilization in Liliaceae. Bull. Coll. Agric. Tokyo **5:** 1902.

11. MAGNUS, W., Studien über die endotrophen Mycorrhiza von *Neottia Nidus-avis* L. Jahrb. Wiss. Bot. **35:** 205–272. 1900.

12. ROSENBERG, O., Physiologische-cytologische Untersuchungen über *Drosera rotundifolia* L. Upsala. 1899.

EXPLANATION OF PLATES XXI–XXIII

PLATE XXI

FIG. 1.—Young ovule showing megaspore mother cell with nucleus just coming out of synapsis; ×1210.

FIG. 2.—Formation of equatorial plate in heterotypic division; ×1210.

FIG. 3.—Homotypic division; ×1210.

FIG. 4.—Row of 4 megaspores; ×520.

FIG. 5.—First division of embryo sac nucleus; two degenerating megaspores seen above it; ×520.

FIG. 6.—Two successive sections (*a*, *b*) showing the 2-nucleate embryo sac; ×520.

FIG. 7.—Four-nucleate embryo sac; ×520.

FIG. 8.—Successive sections (*a*, *b*, *c*) showing the division of the nuclei in the 4-nucleate embryo sac; ×520.

PLATE XXII

FIG. 9.—Upper part of embryo sac showing polar nucleus moving down; ×520.

FIG. 10.—Successive sections (*a*, *b*, *c*, *d*) showing 8-nucleate embryo sac after upper polar nucleus has moved down; ×520.

FIG. 11.—Successive sections (*a*, *b*, *c*) showing mature embryo sac with egg (*e*) and 2 synergids (*syn*), the two polar nuclei (*p*), and 3 degenerating antipodals (*ant*); ×520.

FIG. 12.—Fusion of polar nuclei; ×520.

FIG. 13.—Fertilization; pollen tube (*pt*) seen in protoplasm of one of the synergids and male nucleus fusing with egg nucleus; ×1210.

FIG. 14.—Endosperm after first nuclear division; ×650.

PLATE XXIII

FIG. 15*a*.—Young embryo (*emb*) at the chalazal end of the embryo sac; degenerating antipodals (*ant*) below it; ×520.

FIG. 15*b*.—Division of the lower of the two endosperm nuclei in the same embryo sac; ×520.

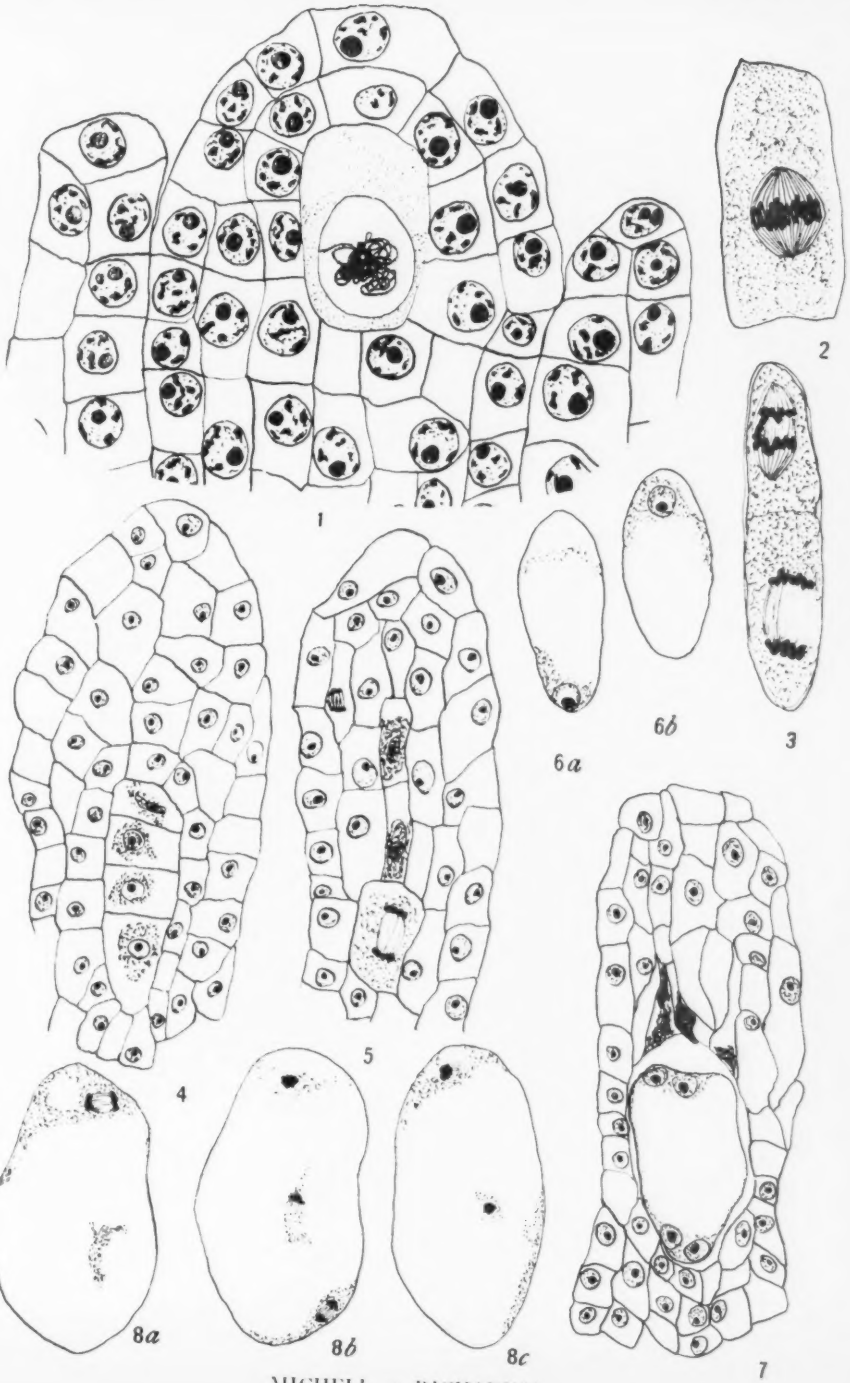
FIG. 15*c*.—Unfertilized egg in the same embryo sac; ×520.

FIG. 16.—Young proembryo; ×520.

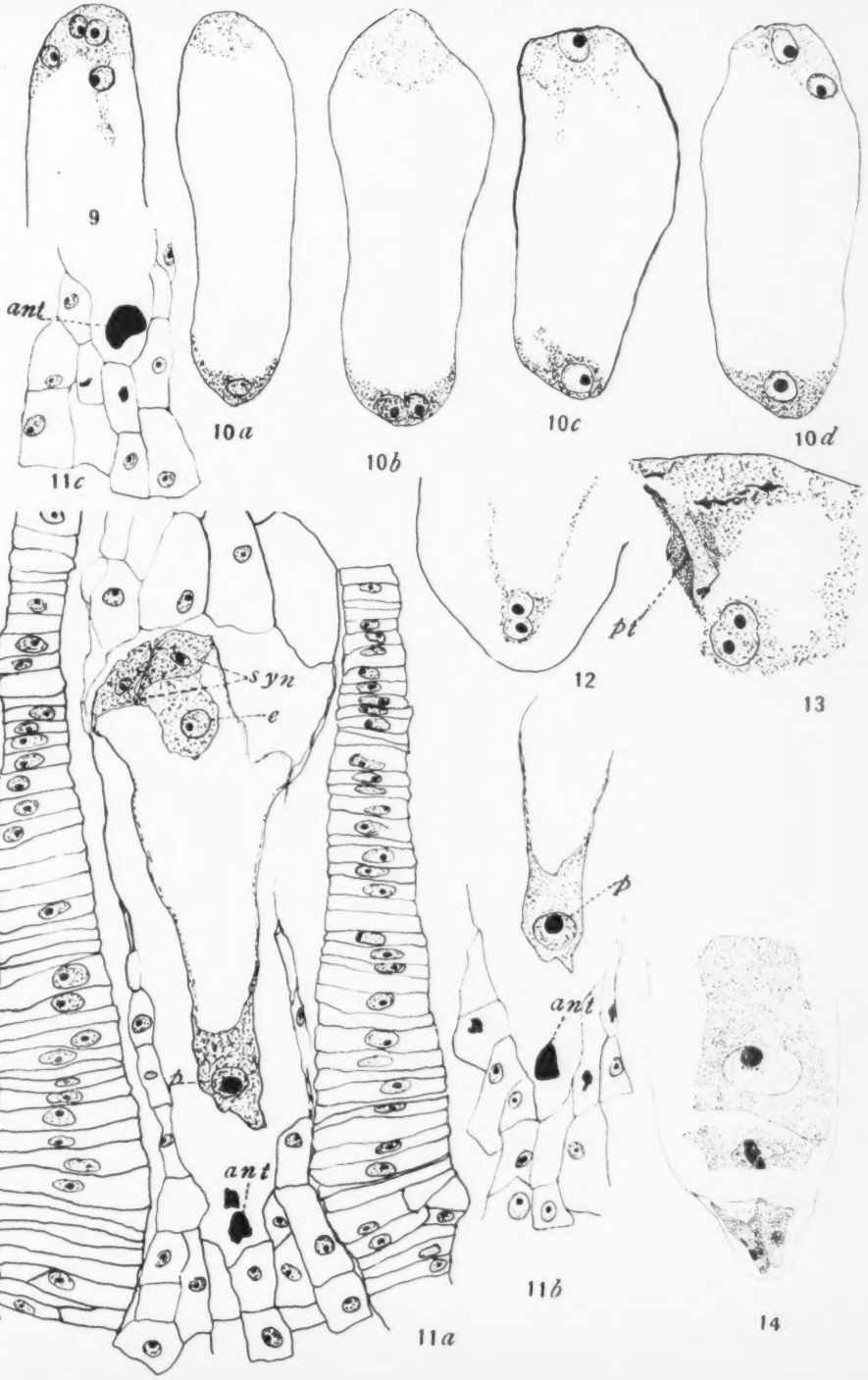
FIG. 17.—Two successive sections (*a*, *b*) showing early stage in endosperm development; ×520.

FIG. 18.—Endosperm showing enlarged basal cells; ×122.

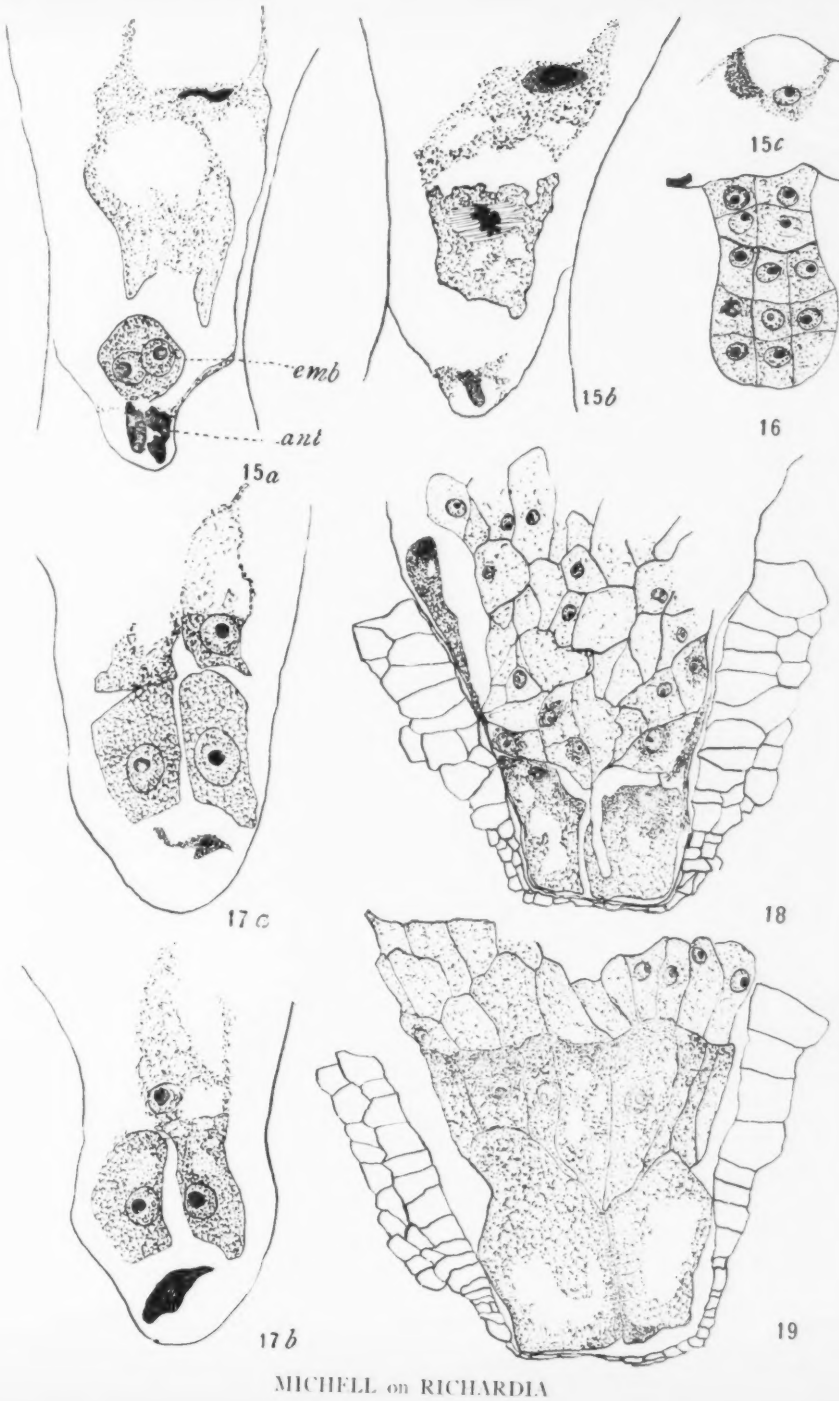
FIG. 19.—Same, in which ordinary endosperm cells have imitated the appearance of the giant cells.



MICHELL on RICHARDIA



MICHELL on RICHARDIA



MICHELL on RICHARDIA

BRIEFER ARTICLES

PREPARATION OF COPY

In response to many inquiries from contributors, the following directions for the preparation of copy for the BOTANICAL GAZETTE are published. If the directions are followed, most of the troubles of contributor and editor will be eliminated.

Preparation of text

Little need be said in reference to the effective method of presentation, selection of material, and style, for in these matters each contributor is a law unto himself. The difference between a clutter of details and a proper laying of emphasis should be recognized. Omitting the obvious is hard for beginners to learn, but they should keep in mind that the audience is made up of professional botanists.

The only practical direction that can be given, therefore, in reference to the preparation of text copy, concerns its form for publication, and this relates to two points. Before the text is prepared, the style of the BOTANICAL GAZETTE should be investigated. Each contributor can find some such paper as he proposes to publish, and should note at least three things: (1) the use of capitals, (2) the use of italics, and (3) the styles of section headings. The chaotic condition of many manuscripts in reference to these features is appreciated by editors but not by contributors. The second point deals with citations, which represent usually the worst feature of a manuscript. In citations the BOTANICAL GAZETTE follows the general style adopted by international agreement, and any list of "Literature cited" shows the style. The purpose of the style is to give the reader exact and full information in the least space. To recast a list of citations is needless editorial labor, when with a little observation the list can be prepared in proper form by the contributor.

Attention may be called to the fact that legends for text cuts should never be written on the figures, but listed at the end of the paper. The printer and engraver are different persons, and their copy must be kept separate. The insertion of figures in the manuscript simply makes work for the editor. The necessary space for editorial annotations should be

kept in mind; manuscripts should not merely be typewritten (never carbon copies), but they should be double spaced and have liberal margins.

Preparation of illustrations

Illustrations in research papers are often disappointing because the author has failed to recognize difficulties which confront the engraver. The following suggestions have been prepared as a general answer to inquiries, and also in the hope that they may enable contributors to secure more effective reproductions of their drawings and photographs. Ten years ago, nearly all illustrations were in the form of plates, but in recent years the text cut has gained in favor, especially in journals printed upon paper suited to the reproduction of photographs and black-and-white drawings. Whether illustrations are to be text cuts or plates, it is recommended that the copy be about twice as large as the desired reproduction. In the case of text cuts, the available space is $4\frac{1}{8}$ inches in width, and, including the legend, $6\frac{1}{2}$ inches in length. The size recommended for plates is $5\times7\frac{1}{2}$ inches, exclusive of the space used by the publisher for the volume number, plate number, and title. These proportions should be maintained, whatever the reduction may be. If the illustrations are to be reduced one-half, the copy for text cuts may be $8\frac{1}{4}$ inches in width, with a maximum length of 13 inches, and plates should be 10×15 inches.

If the separate features of an illustration are to be grouped in a text cut, or are to be arranged in a plate, do this work yourself. Paste the separate figures on a stiff, white cardboard, and paste in the proper places the numbers and any explanatory letters. Suitable numbers and letters for various reductions can be furnished by this office.

The following modes of reproduction have been used by the BOTANICAL GAZETTE: zinc etching, photolithograph, lithograph, heliotype, and half-tone. Of these, only the zinc etching and the half-tone are available for text figures.

Zinc etching.—This type is recommended for graphs, line drawings, and all other black-and-white drawings which do not have extremely fine lines or extremely small dots. For the copy, use smooth, perfectly white paper, preferably Bristol board, and dead black, indelible ink. Lines or dots made after the pen is nearly dry, so that they appear gray in the copy, are sure to disappoint the author. A good example of line drawing, reproduced by zinc etching, at a reduction of one-half, may be found in BOT. GAZ. **42**:412. 1906. For an example combining lines and dots, see **56**:15. 1913; for a graph, see **54**:425. 1912; for a plate, see **35**: *pl.* 7.

1903. For an example of zinc etching, made without reduction, see BOT. GAZ. **61**: *pl. 22*. 1916. The contrast between a zinc etching at a one-half reduction and one reproduced without reduction may be seen by comparing figs. 14 and 13 of the plate just mentioned. The originals for the two figures were drawn in the same style, but fig. 14 was reduced one-half and fig. 13 was reproduced without reduction. Such a procedure involved needless expense, since a negative had to be made from fig. 14, reducing it one-half; then a print from this negative had to be pasted in its place among the original drawings. Contributors often send in graphs on co-ordinate paper, ruled in various shades of red, yellow, or blue. The engraver can "screen" out the colored lines so that they do not appear at all, but he cannot reproduce them satisfactorily. Reproduction of such graphs by the half-tone method is very unsatisfactory. If white paper ruled in black is unobtainable, get a ruling pen and do your own ruling. The graph referred to above was made in this way.

Photolithograph.—This mode of reproduction is good for fine lines and fine dots. The paper must be smooth and perfectly white, and the ink must be dead black. The soft effect of a lithograph can be secured by the printer, who can use an ink of lithograph color. Pencil drawings or washes cannot be reproduced by this method. This is strictly a photographic method, and is popular with investigators who can draw, since it cannot be modified like a lithograph. See BOT. GAZ. **42**: *pls. 19–28*. 1906.

Lithograph.—This method is expensive and somewhat uncertain, since it involves redrawing by the lithographer. A crude drawing will be improved by this method, but if the investigator can draw better than the lithographer, the reproduction will suffer. No really satisfactory lithographs have been made in this country, and there is great delay, sometimes more than a year, in getting them from abroad.

Heliotype.—This method is good for photomicrographs and general work in black and white, but is rather expensive. In the preparation of copy, different colors should not be used, but various shades of black may be obtained by diluting the ink, so that there may be a range from dead black to pencil color. The ink may be used as a wash. It is better not to combine ink and pencil work in a drawing, for while the copy looks well, the ink and pencil do not behave exactly alike when photographed. If photographs or photomicrographs are to be reproduced, use a glossy paper; even then, a skilful squegee will improve the copy. Since the reproduction will lose a little in contrast, use a contrastypaper and

developer. With properly prepared copy, it is never necessary to use this method for line drawing or stippled work.

Half-tone.—This method is almost universally used for the reproduction of photographs of landscapes, models, and portraits. It is also used for photomicrographs. With properly prepared copy it is very satisfactory; but it must be remembered that the screen used by the engraver makes black lines through every white portion, and white lines through every black portion, thus reducing the contrast. Consequently, if the copy is only a fine artistic photograph, the reproduction will be flat and lifeless. In making the negative, use a contrastyplate, develop with a contrasty developer, print on a glossy paper, and squegee the print. Contrast should be so over-emphasized in the copy that the reproduction, rather than the copy itself, shall represent what the author desires. If the figure is to appear as a text cut, $4\frac{1}{8}$ inches in width, it will be much more satisfactory to use a 5×7 copy than a $3\frac{1}{4}\times4\frac{1}{4}$. An enlargement of the copy by this method, or by any other, is wholly unsatisfactory.

THE STRUCTURE OF THE SPIKELET OF APHANELYTRUM

(WITH ONE FIGURE)

In ENGLER and PRANTL'S *Pflanzenfamilien*[1] HACKEL proposes *Aphanelytrum* as a subgenus of *Brachyelytrum*. He bases the subgenus on a single species from Ecuador, *Brachyelytrum procumbens* Hack., differentiating it from *Eubrachyelytrum* by its glumes, minute, "often wanting," and by its thinner, shorter-awned lemmas. The grass was first listed, without description, as *Aphanelytrum procumbens* Hack. in SODIRO'S enumeration,[2] based on HACKEL'S identification of his collections of the grasses of Ecuador. Later HACKEL described[3] the plant as a new genus, discussed its relationship and the structure of its inflorescence, and gave a figure of the supposed spike with three spikelets.

In 1914, among South American grasses received for identification from the Royal Botanical Garden at Petrograd was a specimen collected by JAMESON (no. 168) in Ecuador, which proved to be referable to *Aphanelytrum*. The peculiar spike of three sessile spikelets, the upper two with glumes obsolete, described by HACKEL, is found to be a single 3-flowered spikelet with very long rachilla joints. In the generic description HACKEL says that the 1-flowered, distichous spikelets are alternate and subterminal along the branches of the subsimple panicle,

[1] Nachträge **2**:42. 1897.

[2] Ann. Univ. Quito Ser. **3**:480. 1889.

[3] Oesterr. Bot. Zeitschr. **52**:12. 1902.

the primary branches bearing 3, the secondary 2 or 1 spikelets, the lateral ones sessile, the branchlet with its spikelets forming 2–3-merous

FIG. 1.—*Aphanelytrum procumbens* Hack.: *a*, panicle, nat. size; *b*, spikelet, ×4; *c*, copy of HACKEL's figure.

spikes, of which the axis, articulate above the sterile glumes of the individual spikelets, is produced into a pedicel beyond the uppermost spikelet; that the two glumes are very minute or in the upper spikelets wholly

obsolete, the upper glume (of the lowest spikelet) lying outside of the internode of the axis, both glumes persistent.

In the discussion following the description, HACKEL states that while he had regarded *Aphanelytrum* as a subgenus of *Brachyelytrum* only, further study had convinced him that it was a valid genus. He lays particular emphasis upon the character of the inflorescence:

> The primary branch bears, as it appears constantly, 3 sessile lateral spikelets, the axis extended as a stipelet beyond the uppermost, thus forming a spike, but of so remarkable a structure that I know of no second example among the grasses. Of the two very small glumes which can always be found distinct only on the lowermost spikelet, those of the two upper mostly being wholly aborted, only the lower is found on the same side of the spike axis as the spikelet, the upper being found on the opposite side of the axis. This position is contrary to the conception of this spike as a monopodium, as the spike of the Hordeae, etc., doubtless is; one must rather assume the axis of the spikelet to be the continuation of the spike axis internode below it, and the internode next above to represent a branch in the same direction as that below put forth from the axil of the second glume, the whole spike, then, forming a sympodium. Whether this admits of another explanation later study of more material will decide, but the facts of the case, as the accompanying figure shows, are without doubt. As to the upper members of the spike these can only be understood from analogy to the lower, since in these the glumes are wanting or reduced to minute vestiges.

When this structure is recognized as a single spikelet, the necessity for assuming it to be a sympodium is obviated; the position of the second glume is seen to be the normal one; and, except for the elongated rachilla joints, the spikelet is seen to be in no way anomalous. The minute vestiges of glumes below the upper florets referred to by HACKEL must be the rather prominent callus of the floret.

Among plants recently received from Colombia were several specimens of *Aphanelytrum procumbens* collected along a trail at 3100 meters by Fratres APOLINAIRE and ARTHUR (no. 717). From these specimens the accompanying figure and the following emendation of the generic description are drawn.

APHANELYTRUM Hack. emended

Inflorescence a few-flowered panicle, the remote capillary, flexuous, simple or subsimple branches in fascicles of 2–4 or the upper solitary; spikelets on slender flexuous pedicels, perfect, 2 or 3-flowered, articulate above the minute glumes; rachilla joints capillary, flexuous, from half

to three-fourths as long as the erect or spreading florets, prolonged beyond the uppermost floret; stamens 3.

A specimen of this collection was sent to HACKEL, together with notes on the structure of the spikelet. In reply he writes: "It agrees exactly with the type. . . . . After careful weighing of the evidence I must agree that this explanation is more satisfactory than the one proposed by me, especially because thereby the position of the glumes becomes entirely natural and comprehensible."

The 3-flowered spikelets place this genus in the tribe Festuceae, but where it should be placed in that tribe I am not prepared to say; it is not closely related to any other known genus. In the National Herbarium it is placed for the present between subtribe Meliceae and subtribe Centotheceae. The name *Aphanelytrum*, referring doubtless to invisible glumes, is not so inept, fortunately, since the glumes are very small.—AGNES CHASE, *Bureau of Plant Industry, Washington, D.C.*

CURRENT LITERATURE

BOOK REVIEWS

American trees

Books on trees, most of them of a popular or semi-scientific character, have been produced abundantly within recent years, and many, limiting their scope to some particular region of our country, have successfully met an increasing demand, and have contributed materially to furthering the interests of botanical science. In a recent publication MATHEWS[1] has attempted to include within the limits of a single volume, not only all the trees of the continent that would be likely to interest the informal student of nature, but also the shrubs as well. This is a difficult, if not an impossible task, and it is not surprising that the result, while in many respects excellent, is open to some unfavorable criticism.

One of the first difficulties would naturally be to make a wise selection of the number of species to be included. That this number is large is shown by the inclusion of 25 species of pine, 2 of them European; the same number of oaks; 31 willows; 11 shad bushes; 13 maples; and, still more surprising, 24 species of *Vaccinium*, 15 of *Viburnum*, and 69 of *Crataegus;* while many other genera have been treated with equal generosity. For example, it is hard to see why the white birch should be separated into 7 species and varieties for other than a strictly technical botanical audience. The same criticism would apply with even greater force to *Salix*, *Amelanchier*, and *Crataegus*, especially as the descriptions, although on the whole excellent, are not sufficiently exact and critical to enable even a well-trained botanist to identify a doubtful specimen within these genera. The difficulties are here accentuated by the entire absence of all adequate keys. This is perhaps the most serious fault of the book, and were it not for the extensive series of excellent drawings of leaves and fruit, identification by its aid would be a hopeless task. These drawings, on the contrary, are among the best that have yet appeared, and covering 128 full-page figures and about five times that number of species they seem quite worth the price of the volume. The 66 plates of habit studies of individual trees, 16 of them in color, do not appeal to the reviewer as at all equal to the figures, and could be omitted without serious detriment to other than the possibly artistic value of the book. Other commendable features are the good descriptions of most of the species, the extensive and accurate data as to their distribution, supplemented by some 80 small maps, on each of which the areas

[1] MATHEWS, F. S., Field book of American trees and shrubs. 8vo. pp. xvii+465. *pls. 128*. New York: Putnam. 1915. $2.00.

occupied by 2 to 4 different species are plotted. These things, together with the convenient size of the volume, make the book, in spite of its evident defects, a desirable addition to our non-technical botanical handbooks.—GEO. D. FULLER.

Texts on colloid chemistry

The plant is made in the main of colloids. Notwithstanding this fact, until recently, so far as we have tried to explain its activity on the chemical basis at all, it has been largely in accordance with laws of homogeneous systems. Lately we are coming to realize that the laws of colloids are of first importance in answering many questions concerning the plant, its environment, and the interrelation of the two.

With the translation of the first half of OSTWALD'S *Grundriss der Kolloidchemie*[2] we have an excellent statement of a portion of the principles of the subject available in English. OSTWALD was turned into colloid chemistry by certain problems met in biology, and the translator is an animal physiologist dealing with very fundamental problems in the colloidal side of his subject. These facts should especially interest biologists in the book. It is to be regretted that the second half has not yet appeared in German, and is therefore not available for translation; but one sees the rapidity of the growth in colloid chemistry when he recognizes that the first half passed through three editions without opportunity for writing the second half.

OSTWALD, with his attractive way of presenting a subject, hardly needs an introduction to an American scientific audience, following his recent extensive lecture tour in this country. The translator says "WOLFGANG OSTWALD'S writings represent in colloid chemistry what those of CHARLES GERHARDT represent in organic, JUSTUS LIEBIG in agricultural, and WILHELM OSTWALD in physical chemistry." Notwithstanding the fact that many phases of the subject of greatest interest to physiologists are still to be treated in the second half, the volume is a much-needed reference book for plant workers.

TAYLOR'S[3] book upon colloids is a far less exhaustive statement, but offers a good general outline of the subject. Part I (163 pp.) deals with general properties of colloids; part II (56 pp.), with methods of preparation; part III (42 pp.), with surface phenomena or adsorption; and part IV (56 pp.), with applications of colloid chemistry (semi-colloids, dyeing, tanning, soil, purification of sewage, and applications to biology). Those interested only in the general principles of the subject will find this little book most satisfactory.

[2] OSTWALD, WOLFGANG, A handbook of colloid chemistry. 1st Eng. ed. translated from 3d Ger. ed. by MARTIN H. FISCHER, with the assistance of R. E. OESPER and L. BERMAN. 8vo. pp. xii+278. Philadelphia: P. Blakiston's Son & Co. 1915. $3.00.

[3] TAYLOR, W. W., The chemistry of colloids and some technical applications. pp. vi+328. New York: Longmans, Green, & Co. 1915. $2.00.

The reviewer, however, feels that there is no other statement on colloids so significant and helpful to biologists as the chapters dealing with this and related topics in HÖBER's[4] work (chaps. vi, vii, and many points in later chapters).—WM. CROCKER.

An elementary textbook

The textbooks of botany for elementary students are multiplying so rapidly that every conceivable method of presentation will soon be available. It is no longer a question as to the facts to be presented, but as to the method of presentation. An interesting organization of the fundamental facts of botany for the benefit of elementary students is the recent text by THODAY,[5] who says that the book is intended primarily for use in connection with the "Senior Cambridge local examinations." No previous knowledge of botany is assumed, so that the presentation is intended to be strictly elementary.

Without questioning the facts, the interesting feature of the book is its testimony as to the requirements in botany in the examinations referred to. The book is divided into five sections, whose titles sufficiently indicate the contents. After an introductory chapter, pointing out such conspicuous organs of plants as may be shown by a comparison of sunflower, grass, dandelion, and horse-chestnut, the first section deals with "The functions of plant organs" and "The food of plants." The first contact, therefore, is physiological, before any knowledge of structure has been developed. This follows in the second section, under the title "Form and structure," which is an outline of anatomy. The third section bears the title "Reproduction," but it is merely reproduction by seed plants, dealing with flowers, fruits, seeds, and germination. "The classification of plants" is the title of the fourth section, and this also is restricted to seed plants. The principal chapter is entitled "Evolution and the principles of classification as illustrated by the buttercup family." This section is really an introduction to some of the important families of angiosperms. The last section is ecological, under the title "Plants in relation to their environment," the five chapter headings illustrating the treatment as follows: fitness, trees, climbing plants, water plants, the distribution of plants and the factors which govern it.

The notable feature of the book, aside from the order of presentation, is the elimination of all plants except flowering plants. It is not a question as to the importance or unimportance of the cryptogams, but as to any intelligent appreciation of flowering plants apart from some perspective of the plant kingdom as a whole.—J. M. C.

[4] HÖBER, R., Physikalische Chemie der Zelle und das Gewebe. pp. xviii+808. Berlin: Wilhelm Englemann. 1914. $5.50.

[5] THODAY, D., Botany; a textbook for senior students. 8vo. pp. xvi+474. *figs. 205*. Cambridge: University Press. 1915. 5*s*. 6*d*.

Plant histology

The third edition of CHAMBERLAIN'S[6] *Methods in plant histology* has been entirely rewritten, and so much new matter both in text and illustrations added that it is essentially a new book. A chapter on the making of photomicrographs and lantern slides has been added, and the directions are so lucid and complete that anyone should be able to do good work, even with inexpensive and improvised apparatus.

Perhaps in no field of microtechnique has the advance been greater than in the paraffin method during the past ten years. The later methods are fully set forth. The chapter on special methods has been much enlarged and brought up to date. Many of the time-honored formulae and methods have disappeared; only those which have stood the most rigid tests have survived in the third edition. Many new methods and formulae are presented for the first time.

Much attention has been given to collecting and keeping material alive in the laboratory. The directions for collecting, killing, and fixing material for research are most thorough, for the author keenly realizes how difficult it is to get material properly prepared for critical research. His experience has been such that he knows that many otherwise competent collectors entirely fail to understand or can be made with difficulty to realize the supreme importance of properly killing, fixing, and preserving material intended for research.

Specific directions are also given for making such preparations as are needed by teachers and those who wish to get a comprehensive view of the plant kingdom from the lowest to the highest forms. The book is indispensable to those who wish to be in touch with the latest advances in modern microtechnique.—W. J. G. LAND.

MINOR NOTICES

Amino acids.—UNDERHILL'S[7] little book on the physiology of amino acids is an interesting and simple general statement of the present status of our knowledge on this subject. The chapter headings give an idea of the nature and scope of the treatment of the topic. They are as follows: (1) The proteins and their derivatives, the amino acids; (2) digestion and bacterial activity in relation to the amino acids; (3) the absorption of proteins and amino acids; (4) in what forms does ingested protein enter the circulation; (5) theories of protein metabolism; (6) the further fate of amino acids; (7) the amino acids in relation to the specific dynamic action of proteins; (8) the amino acids and simpler nitrogenous compounds as foodstuffs; (9) the specific rôle of amino acids in nutrition and growth. The book is most attractively written and

[6] CHAMBERLAIN, CHARLES J., Methods in plant histology. pp. xi+314. The University of Chicago Press. 1915.

[7] UNDERHILL, F. P., The physiology of amino acids. pp. 169. *figs. 13.* Yale University Press. 1915. $1.35.

thoroughly suited to the audience addressed. While the subject is treated entirely from the side of mammalian physiology as a basis of comparison, the book bears much of interest to plant physiologists. The limited power of mammals to manufacture amino acids, especially lysine and tryptophan, the inadequacy of certain plant proteins (zein and gliadin) as a nitrogen source for mammals because of the absence of one or both of these amino acids, and the idea that amino acids play a specific rôle in metabolism and perhaps growth, aside from their use as source of energy and building material, are all suggestive to the plant physiologist, and contrast with the situation more generally met by him.—WM. CROCKER.

A new manual.—PIPER and BEATTIE[8] have published a manual of the flora of the region described as "lying between the summit of the Cascade Mountains and the Pacific Ocean from the 49th parallel of latitude across the southern portion of Vancouver Island, south to the headwaters of the Willamette River." There are four life zones represented: humid transition zone, including the great forests of Douglas spruce; Canadian zone, not sharply limited; Hudsonian zone, indicated by subalpine fir, Alaska cedar, black hemlock, and white-bark pine; and arctic zone, consisting of the alpine flora above timber line. It is a most interesting floral region, not hitherto represented in a suitable manual. The material upon which the work is based is mainly to be found in the herbarium of the State College of Washington.

The usefulness of a manual can be judged only by its use; but so far as organization and appearance go, this manual promises to be all that can be desired. The size of the volume indicates a rich and varied flora, and the summary states that 1617 species and subspecies are presented, representing 550 genera and 100 families. New species are described in *Arctostaphylos*, *Godetia*, *Panicularia*, *Populus*, and *Solidago* (2), and 14 new combinations are proposed. A useful glossary and a full index complete the volume.—J. M. C.

Botanical technique.—The second volume of the *Praktikum* of MÖBIUS[9] deals with thallophytes, bryophytes, pteridophytes, and gymnosperms. The descriptions and directions are in general good, but the quality of the illustrations is very variable. Some are excellent, others are so faithfully drawn that the carelessness of the technician is very apparent, as shown by the figure of a cross-section of the stem of *Lycopodium complanatum*. A figure of a young antheridium of *Pellia epiphylla* shows that the illustrator did not know he was drawing from an oblique section. In any text, especially one intended for beginners, accuracy and clearness of statement should be paramount. In addition to an intimate knowledge of the subject, an author should also be

[8] PIPER, CHARLES V., and BEATTIE, R. KENT, Flora of the northwest coast. 8vo. pp. xiii+418. Lancaster (Pa.): The New Era Printing Co. 1915. $1.75.

[9] MÖBIUS, M., Mikroskopisches Praktikum für systematische Botanik (II). 8vo. pp. v+314. *figs. 123*. Berlin: Gebrüder Borntraeger. 1915.

able to interpret a section correctly and to know when there is a glaring fault in his technique.—W. J. G. Land.

Illustrations.—A series of lectures dealing with the illustration of botanical papers was delivered at the University College, London, in 1913, by T. G. Hill. In response to various requests, these lectures are now published in book form.[10] The various forms of intaglio, plane surface, and relief printing are described, and their limitations noted. Suggestions are given for the preparation of copy suited to the various types of reproduction. The descriptions of processes are interesting, and, combined with the practical hints, should enable investigators to furnish more effective copy. There is no effort to give instruction in drawing.—C. J. Chamberlain.

North American Flora.—The third part of Vol. 17 continues the presentation of the Poaceae, and includes the genus *Panicum* by Hitchcock,[11] who recognizes 211 species distributed among 46 tribes. No new species are described, but it is interesting to note that Hitchcock's name is associated with 32 of the species. Other diligent students of the species have been Nash (30 species), Scribner (25 species), and Ashe (16 species).—J. M. C.

NOTES FOR STUDENTS

Anthocyanins.—Willstätter[12] and his students have made an extensive study of the anthocyanins of various flowers and fruits. The findings are certain to prove of great importance to plant workers, especially breeders and physiologists. The work puts this previously little understood group of plant pigments among those most thoroughly worked. All such matters as methods of extraction, purification, and quantitative estimation, general chemical constitution, general chemical characters, empirical and structural formulae, and

[10] Hill, T. G., The essentials of illustration. 8vo. pp. xii+95. London: Wesley & Son. 1915.

[11] North American Flora **17**:part 3. pp. 197–288. Poales: Poaceae (pars), by G. V. Nash and A. S. Hitchcock. New York Botanical Garden. 1915.

[12] Willstätter, R., Über Pflanzenfarbstoffe. Ber. Chem. Gesells. **47**: 2831–2874. 1915; Willstätter, R., and Nolan, T. J., II. Über den Farbstoff der Rose. Ann. Chem. **408**: 1–14. 1914; Willstätter, R., and Mallison, H., III. Über den Farbstoff der Preiselbeere, *ibid.* 15–41; Willstätter, R., and Bolton, K., IV. Über den Farbstoff der Scharlachpelargonie, *ibid.* 42–61; Willstätter, R., and Mieg, W., V. Über ein Anthocyan des Rittersporns, *ibid.* 61–82; Willstätter, R., and Zollinger, E. H., VI. Über die Farbstoffe des Weintraube und der Heidelbeere, *ibid.* 83–109; Willstätter, R., and Martin, K., VII. Über den Farbstoff der *Althaea rosea*, *ibid.* 110–112; Willstätter, R., and Mieg, W., VIII. Über den Farbstoff die wilder Malve, *ibid.* 122–135; Willstätter, R., and Nolan, T. J., IX. Über den Farbstoff die Päonie, *ibid.* 136–146; Willstätter, R., and Mallison, H., X. Über Variationen der Blütenfarben, *ibid.* 147.

the cause of variation in the color of flowers bearing these pigments, have been established or made very probable.

The anthocyanins are characterized as nitrogen-free substances of amphoteric nature. Their strong basic character is due to the fact that with acids they form oxonium salts with tetravalent oxygen. This character forms the best basis for their isolation and purification, since on this basis they form good crystalline bodies with various organic and inorganic acids. The chlorides were most used. The acid character of the anthocyanins is due to their polyphenolic nature. This was formerly used as the basis for isolation by the precipitation as lead salts, but it is undesirable, since many other substances fall out with the pigments. The acid salts are red, the neutral anthocyanins violet, and the alkali salts (for such anthocyanins as form them) are blue. In neutral and still more frequently in alkaline condition the anthocyanins become colorless. This is due to an isomerization, and not to a reduction, as is generally assumed. This is suggestive in explaining the loss of color by flowers under certain conditions.[13] Upon acidulation the color returns. In plants the anthocyanins exist entirely or nearly so as glucosides. Brief heating of the anthocyanins with 20 per cent HCl splits them into glucose and the corresponding anthocyanidins. The following are some of the studied anthocyanins and their splitting products.

Anthocyanin	Isolated from	Anthocyanidin +
Cyanin	Centaurea cyanus	Cyanidin
($C_{27}H_{31}O_{16}Cl\ 2\frac{1}{2}H_2O$)		($C_{15}H_{11}O_6Cl$ + 2 glucose)
Pelargonin	Pelargonium zonale	Pelargonidin
($C_{27}H_{31}O_{15}Cl\ 4H_2O$)		($C_{15}H_{11}O_5Cl$ + 2 glucose)
Delphinin	Delphinium consolida	Delphinidin
($C_{41}H_{39}O_{21}Cl\ 12H_2O$)		($C_{15}H_{11}O_7Cl$ + 2 glucose + 2 p oxybenzoic acid)
Önin	Deep colored wine grape	Önidin*
($C_{23}H_{25}O_{12}Cl\ 4H_2O$)		($C_{17}H_{15}O_7Cl$ + 1 glucose)
Malvin	Wood malva	Malvidin*
($C_{29}H_{35}O_{17}Cl\ 8H_2O$)		($C_{17}H_{15}O_7Cl$ + 2 glucose)

* Önidin and malvidin are dimethyl ethers of delphinidin.

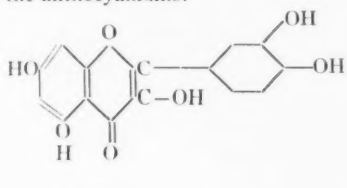
Several physical constants (crystal shape and color, melting or decomposition points, solubility in various reagents, isomerization with soda, and reaction with iron chloride and iron picrate) of these anthocyanins and anthocyanidins have been tabulated. These, with the methods of extraction and purification, put significant methods into the hands of plant breeders of moderate chemical training.

Variations in the color of flowers bearing these pigments are due to one or more of four causes: (1) various anthocyanins in the same flower or species; (2) great variation in the anthocyanin content; (3) chemical reaction of the

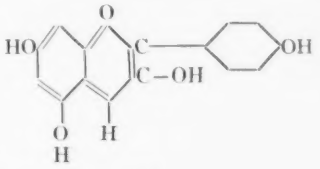
[13] FITTING, H., Über eigenartige Farbanderungen von Blüten und Blütenfarbstoffen. Zeitschr. Bot. 4:81–106. 1912.

cells; (4) mixtures with yellow pigments. The following are a few illustrations of the significance of these factors: (1) The dark purple-red flowers of *Centaurea cyanus* bear cyanin, the rose-red flowers pelargonin; a violet-red variety of *Pelargonium* bore mainly cyanin with a slight amount of pelargonin. This is said to be the first plant in which a mixture of anthocyanins was found in the same flower. (2) The bright red and dark red garden rose both bear cyanin with a great difference in concentration. The flower of the field *Centaurea cyanus* contains 0.65–0.70 per cent dry weight of alkaline cyanin, while a dark purple double garden variety bears 13–14 per cent. (3) Blue flowers of *Centaurea* contain potassium salts of cyanin, violet flowers of *Delphinium* neutral delphinin, and scarlet flowers of *Pelargonium* tartrate of the anthocyanin. (4) The following yellow pigments may act with anthocyanins in determining the flower color: (*a*) neutral carotinoids, carotin and xanthophyll; (*b*) the glucosidic flavone pigments; (*c*) the little-known anthochlors of the cell sap. It is found that the green color shown by a crude extract of anthocyanin of alkaline reaction results from the mixture of the blue of the alkali salts of the anthocyanin and a yellow pigment. Some of the flavone dyes, also the pseudobases of anthocyanin, are almost colorless in neutral and acid solutions, but intensely yellow in alkaline solution. This is suggestive to breeders dealing with yellow and white flowers.

We are coming to know something of the substances from which the anthocyanins originate, and the reactions by which they are produced. There are many facts to suggest a close relationship between the yellow pigments of plants of the favone or flavonol type and the anthocyanins. COMBES[14] has made probable that this is a genetic relationship in obtaining anthocyanin by the reduction of yellow pigments extracted from plants; and EVEREST[15] brings still more evidence for the view that the anthocyanins originate from the flavonol yellow pigments by a simple process of reduction. He has produced anthocyanins by reducing flavonol glucosides and similarly anthocyanidins by reducing the sugar-free flavonol derivatives. The following formulae express his idea of the relation between the yellow flavonols and the chlorides of the anthocyanidins:



Quercetin,
a flavonol derivative



Cyanidin chloride,
an anthocyanidin

[14] Compt. Rend. Acad. Sci. Paris **158**: 272–274. 1914; Ber. Deutsch Bot. Gesells. **31**: 570–578. 1914.

[15] Jour. Genetics **4**: 361–367. 1915.

These formulae will also serve to give an idea of the constitution of the anthocyanidins. The flavonols and flavones are well known yellow pigments of plants. Our thorough knowledge of the chemistry of these pigments is partly due to their extensive use in the dyeing industry. WHELDALE[16] has suggested that anthocyanins originate from the flavonol glucosidic pigments by a process of hydrolysis followed by oxidation, and she questions EVEREST's ideas as set forth above, so far as they apply to the origin of anthocyanins in plants, since the drastic reagents used by EVEREST are not available for the plant. It would seem that her protest is rather poorly grounded.

Almost every point established concerning the anthocyanins is of great immediate significance to plant breeders and physiologists. WILLSTÄTTER and his students have done much to put our knowledge of this group of pigments on solid foundations, as they previously did for the pigments of the chloroplast.—WM. CROCKER.

Anatomy of Isoetes.—LANG,[17] in continuing his studies of *Isoetes*, has analyzed the stele of *I. lacustris*, with the help of apical development. The contradictory interpretations of the stem of *Isoetes* have arisen from complications due to the occurrence of crowded leaves upon a very slightly elongating axis, accompanied by the continued growth of the cortex. The summary of the analysis is as follows, proceeding from within outward: (1) central column of primary xylem (the strictly cauline region of the stem); (2) peripheral zone of xylem, consisting of bases of leaf traces connected with the central cylinder and radially arranged xylem between the entering leaf traces; (3) parenchymatous xylem sheath, continuous with similar region in leaf trace; (4) primary phloem, continuous with phloem of leaf trace; (5) secondary prismatic tissue, consisting of tracheids, sieve tubes, or parenchyma; (6) meristen of secondary prismatic tissue; (7) cortical tissue. LANG states that such an analysis of the stele of *Isoetes* "not only affords points for comparison with the Lepidodendreae, but promises to be of interest from the standpoint of general stelar morphology."—J. M. C.

Espeletia.—This is a genus of the Asteraceae, restricted so far as known to the high cordilleras of Colombia and Venezuela. The genus is among the most conspicuous of the composites, the leaves and inflorescences in most of the species being closely invested by long wool. The genus has just been revised by STANDLEY,[18] who recognizes 17 species, 6 of which are described as new.—J. M. C.

[16] Jour. Genetics **4**:369–376. 1915.

[17] LANG, WILLIAM H., Studies in the morphology of *Isoetes*. II. The analysis of the stele of the shoot of *Isoetes lacustris* in the light of mature structure and apical development. Mem. and Proc. Manchester Lit. and Phil. Soc. **59**:29–56. *pls. 4. figs. 7.* 1915.

[18] STANDLEY, PAUL C., The genus *Espeletia*. Amer. Jour. Bot. **2**:468–485. *figs. 6.* 1915.